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ON THE *IN VITRO* METABOLISM OF LABELED GLUCOSE IN NORMAL AND PSORIATIC SKIN SLICES

BY

C.-G. HERDENSTAM

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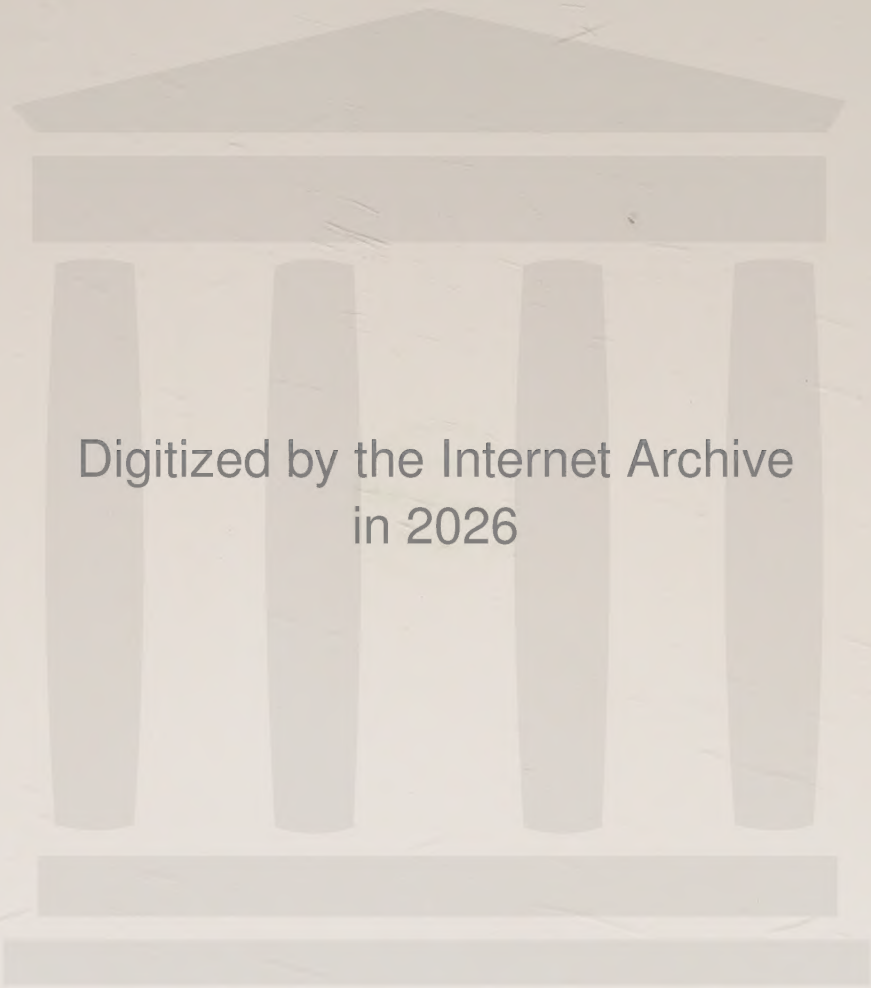
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VOL. 42. SUPPLEMENTUM 47

FROM THE DEPARTMENT OF DERMATOLOGY, SÖDERSJUKHUSET, STOCKHOLM, SWEDEN
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HÅKAN OHLSSONS BOKTRYCKERI

To my mother

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INTRODUCTION

The pathogenesis of many important skin disorders, including psoriasis, which probably are associated with a metabolic disturbance, is still unknown in spite of the fact that skin specimens are readily available for morphological, biophysical or biochemical analyses. Moreover, the basic metabolic pattern of human skin has not yet been completely elucidated. Our limited knowledge in this field seems to be due partly to the lack of an appropriate method for studying the metabolism of human skin.

The quantitative, two-dimensional radiochromatographic technique, developed by *Chain et al.* (6) at the Istituto Superiore di Sanità in Rome, has been used for several years with great success in metabolic studies on different animal biopsy specimens such as rat diaphragm muscle (6), rat liver and brain slices (3, 5, 16) rat pituitary gland (4), rat adipose tissue (7) and different parts of the rabbit brain (17).

The purpose of the present investigation, where the radiopaperchromatographic technique of *Chain et al.* (6) was applied for the first time to human biopsy material, is primarily to introduce a new and apparently valuable method into the field of dermatology, in order to obtain a better understanding of the biology of the skin. Furthermore, this work is intended as a contribution to our knowledge of the pathogenesis of psoriasis.

ABBREVIATIONS AND DEFINITIONS

μC: Microcurie = $3.7 \cdot 10^4$ atomic disintegrations per second.

CPM: Counts per minute.

Specific activity: Radioactivity per weight unit of radioactive material.

HMP-shunt: Hexose monophosphate shunt.

EMP: Embden-Meyerhof pathway.

GOT: Glutamic oxaloacetic transaminase.

TTC-reaction: Histochemical proof of succindehydrogenase activity by means of a solution of triphenyltetrazoliumchloride (TTC).

RQ: Respiratory quotient.

P: Probability that the differences between the means are due to chance alone.

$\bar{x}$: Mean value.

S.e.m.: Standard error of the mean.

TPNH: Reduced triphosphopyridine nucleotide.

CHAPTER I

SURVEY OF PREVIOUS BIOCHEMICAL STUDIES ON PSORIASIS

In a previous work (34) the author gave a review of the theories advanced hitherto concerning the etiology and pathogenesis of psoriasis. This publication included, moreover, most of the biochemical data on this skin disease published during recent decades. Consequently, the survey of the literature given in this chapter will be limited to those papers which are directly related to the present investigation.

ON PREVIOUS METHODS OF EVALUATING THE CELLULAR ACTIVITY AND THE METABOLISM IN PSORIATIC SKIN

The dominant feature in the psoriatic skin process is the high cellular activity in the epidermis, which is due mainly to the rapidly proliferating epidermal cells. The clinical manifestations of this hyperactivity are the thickening of the affected skin areas and the increased scaling. The microscopic examinations of the psoriatic lesion which were made by several investigators (13, 32, 49), revealed a fairly uniform picture: A thickening of the epidermis with loose lamellar hyperkeratotic scales, alternating with islands of parakeratosis containing nuclear remains; the absence of a granular layer, a proliferation of the prickle cell layer and an elongation and broadening of the rete pegs, where the akanthotic proliferation and the increased mitotic activity are most evident. Moreover, the existence of markedly dilated and tortuous capillaries in the psoriatic lesion has been established in different ways. Recently this has been done by applying an alkaline phosphatase colouring technique (32, 35). Hence, the blood supply of the psoriatic plaque may be very abundant, which seems to be in agreement with the assumption of a high cellular activity in the diseased skin.

The increased metabolic rate of the psoriatic skin lesion has hitherto been demonstrated either by measuring the oxygen consumption or by histochemical investigations. Thus, an increased oxygen uptake of the psoriatic skin was reported when applying the Warburg technique (74). More recently, a considerably increased oxygen utilization and a conspicuous glycolysis in psoriatic lesions were observed by *Stuttgen* (79), using the same technique.

Histochemical analyses of psoriatic skin specimens, where the TTC reaction was used, have revealed an increased succinic dehydrogenase activity (45, 74, 82), which implies an increased cellular metabolism. This might be explained by the fact that the energy required for the increased cellular activity is substantially obtained through the Krebs cycle, in which part of the reactive chain is formed by dehydrogenation of succinic acid

into fumaric acid. According to *Braun-Falco* (12), there is a surprisingly good correlation between the oxygen consumption and the succinic dehydrogenase reaction. The increased enzyme activity is, however, not specific for psoriasis but corresponds to the extent of the akanthotic proliferation.

Other enzymatic manifestations of the intense metabolic activity of the psoriatic lesion are the increased level of β-glucuronidase (11), arginase (64), GOT (86), acid phosphatase (73) and phosphorylase and glucosidase (78); the last 3 enzymes mentioned play a dominant role in the interconversion of glucose and glycogen. The very high potassium level in the psoriatic skin, reported by *Reinberg et al.* (59), also indicates an increased proliferating capacity, since potassium is indispensable to the anabolism of proteins, and hence, to the building-up of the cell structure. Furthermore, the beneficial effect of aminopterin, a cytotoxic agent blocking the nucleic acid metabolism, previously reported in the treatment of psoriasis (28, 33, 57, 58), strongly suggests that the pathogenesis of this disease involves an increased, proliferative cellular activity in the epidermis.

By means of histochemical methods it has moreover been claimed that the cholinesterase activity is greatly decreased in the psoriatic lesions (82). Since acetylcholine probably is one of the most important vasodilator metabolites in the skin (65), the above-mentioned capillary dilation in psoriasis could thus be explained on the basis of a decreased decomposition of acetylcholine.

Finally, electron microscopic investigations have recently revealed that the cytoplasm of psoriatic cells contains numerous mitochondria and that the endoplasmic reticulum with attached ribosomes is strongly developed (87). These findings definitely indicate an increased metabolic activity of the epidermal cells in psoriasis.

In spite of the positive results obtained by investigations of this kind, our knowledge about the metabolism in normal and psoriatic skin has, however, remained most unsatisfactory until recent years, when the modern isotope technique was introduced in the field of dermatology. A pioneer work of this kind was presented in 1955 by *Nicolaides and Rothman* (53), who demonstrated the epidermal biosynthesis of sterols and fatty acids by incubation of human skin slices, obtained from an amputated leg, in a C^{14}-acetate medium. Kimmelstiel (44) studied the metabolism of C^{14}-glucose by incubating human skin slices, obtained at autopsy, in different C^{14}-glucose mediums (Glucose-U-C^{14}, glucose-1-C^{14} and glucose-6-C^{14}). In this way the classical pathways of the glucose metabolism could be clearly demonstrated in human skin. Thus, it could be proved, that C^{14}-glucose was abundantly utilized for glycolysis, for production of carbon dioxide, and for incorporation into fatty acids and sterols. Incubation experiments conducted with specifically labeled glucose, demonstrated moreover the existence and significance of the hexosemonophosphate shunt in human skin. This last finding supports the assumption, that oxidation of glucose via this pathway might constitute the more abundant source of TPNH in the skin. A disadvantage of this interesting study was, however, that the technique employed did not give any information either on the conversion of glucose into amino acids or on the formation of proteins. Besides, this investigation was not performed on skin slices from living individuals.

As previously mentioned, *Chain et al.* (6), at the Istituto Superiore di Sanità in Rome, developed a very suitable method for a more complete study of the metabolism of C^{14}-glucose in biopsy materials. Furthermore, most of our present knowledge of the mode of action of insulin is founded on a series of investigations carried out by *Chain et al.* (15) using the quantitative radiopaperchromatographic technique, upon which the present study is based.

SOME REMARKS ON THE KERATINIZATION PROCESS IN NORMAL AND PSORIATIC SKIN

The transformation of living epidermal cells into horny material is called the keratinization process. As pointed out by *Rothman* (65), this process represents one of the most important facets of the biology of the skin. The normal keratinization does not only include the transformation of cytoplasmic proteins into keratin fibrils, but also a complete disintegration of the keratinizing cell, including decomposition of both cytoplasm and nucleus. Hence, conditions such as psoriasis, where the faulty keratinization plays a dominant role, are characterized by formation of anomalous keratin, intimately correlated to disturbances of the cellular decomposition.

Chemically, the keratin represents a macromolecule composed of long, spiral-shaped polypeptide chains held together by four different types of cross-linkages; disulfide bonds, salt linkages, hydrogen bonds and van der Waal's forces. The disulfide bridges, formed by the oxidation of the sulfhydryl groups belonging to two cysteine residues in adjacent polypeptide chains, are the strongest linkages, and are mainly responsible for the chemical and mechanical resistance of the keratin layer of the skin. Moreover, the elasticity of the keratin fibrils depends on the existence of disulfide cross-linkages between the polypeptide chains.

Until recent years, the SS-bridges of normal adult keratin have been commonly supposed to originate from oxidated SH-groups, initially belonging to cysteine molecules in the Malpighian layer of the epidermis. The finding of SS-containing keratin precursors in the living cells of the Malpighian layer (23) has, however, supported the hypothesis that the keratinization process begins already in this layer. According to Magnus (50), the gradual disappearance of the sulfhydryl groups during normal keratinization might be explained by a progressive folding of the polypeptide chains, thus diminishing the number of reactive SH-groups. Analogously, the large amount of sulfhydryl groups in psoriatic scales, indicated by several histochemical investigations (14, 21, 67, 75, 76, 91) has been regarded as a sign of incomplete keratinization, primarily owing to the unfolding of the protein chains (41, 50). This concept cannot be accepted, however, without raising certain objections. Firstly, the remarkably low cysteine content in psoriatic scales, recently reported (90), suggests the presence of other sulfhydryl compounds in the psoriatic skin. Second, the histochemical method commonly used for estimation of the SH-activity is not entirely specific (70). Third, spectromicrophotometric determinations of the sulfhydryl level of stratum corneum have revealed only small differences between normal and psoriatic skin (77). Fourth, an ingenious study

on the structure of keratin by means of low-angle X-ray diffraction has proved that the keratinization process involves a coating of the keratin fibrils with a lipid layer (80). The increased amount of SH-groups in psoriatic scales could consequently be due merely to an incomplete lipid coating or to a defficient aggregation of the protein. Moreover, it has been emphasized by *Flesch & Esoda* (21) that the chemical deviations in psoriatic scales, including a high sulfhydryl content, are restricted to the water-extractable part of the horny layer. This observation clearly indicates that the major changes in the keratinization might be due to a faulty cellular decomposition and not exclusively to the formation of anomalous keratin fibrils.

This theory is further supported not only by the similar amino acid and peptide patterns, obtained by chromatography, after hydrolysis of normal and psoriatic keratin (39, 48, 62) but also by the almost identical sulfur content in the keratin residue of normal and of psoriatic skin after elution of the water-extractable components (26). Therefore, in spite of the ultrastructural abnormalities in the psoriatic keratin, clearly demonstrated by *Swanbeck* (80), it seems reasonable to stress once more the cytochemical deviations in the psoriatic epidermis, obviously correlated to a faulty cellular decomposition.

The defective nuclear disintegration in the psoriatic epidermis is primarily reflected in the accumulation of the following water-soluble components:

1. Pentoses (22, 27)
2. Free, inorganic phosphates (81)
3. Soluble proteins: fibrous proteins, glycoproteins and nucleoproteins (61)
4. Aromatic amines (38)

Besides these peculiarities, the water extract of psoriatic scales is characterized by a marked reduction in the amount of polypeptides (55) and a high SH-activity in combination with a low amino nitrogen level (21).

Psoriatic keratin is also said to contain an increased amount of phospholipids (41). This finding probably indicates that the phospholipids have been incompletely catabolized. Contradictionally, it has also been claimed that the accentuated keratinization in psoriasis is associated with an increased breakdown of phospholipids (37). This assumption is confirmed by the finding of large quantities of free choline in the psoriatic keratin (54, 72).

To summarize, previous investigations in this field have indicated an increased activity of the epidermal cells in psoriasis, associated with a defective keratinization. This implies an accentuated formation of abnormal keratin fibrils characterized by faulty aggregation of the tonofilaments intimately correlated to an impaired cellular decomposition; the latter phenomenon seems to account for the principal chemical deviations, hitherto reported, in the psoriatic skin.

CHAPTER II

CLINICAL MATERIAL AND COLLECTION OF SKIN SAMPLES

CLINICAL MATERIAL

The clinical material, which is summarized in Table 1, was composed of three test groups. The first one included 13 apparently healthy volunteers without any clinical signs of skin disease, diabetes or any other metabolic disease. The second group consisted of 11 patients suffering from chronic psoriasis with nummular skin lesions affecting the extremities, the scalp or the trunk but *not* the gluteal region from which the skin specimens were taken. This material was designated as "uninvolved skin". The third group included 13 patients suffering from the same type of chronic psoriasis with plaque formation also over the gluteal region. Some of the patients in group 2 were also included in the last group, which implies that with regard to these last patients skin samples were taken both from uninvolved and diseased skin. All of the psoriatics had suffered from psoriasis for many years, on an average for 23 years. Most of them had been hospitalized on one or more occasions. The skin lesions were generally very extensive. Before the investigation every type of local treatment, except white petrolatum, was discontinued for at least 3 days. In order to avoid the influence of a sex factor, only male subjects were chosen for the present study.

COLLECTION OF SKIN SAMPLES

Equipment

Brown Electro-Dermatome with changeable blades. The instrument is originally used for surgical skin grafts. The required thickness of the graft is obtained by using the 2 micro adjustment knobs on the instrument. The thickness of the graft is uniform throughout with no irregularity in cutting. The cutting blade has a speed of 8.000 cutting strokes per minute. The width of the graft is accurately selected by an adjustment device on the dermatome. The instrument is driven by a small electric motor connected to a flexible driving shaft (Fig. 1).

Procedure

In each case the skin biopsies were taken from the gluteal region by means of the Brown Electro-Dermatome. Before the biopsy, the skin was carefully cleaned with a liquid antiseptic soap (Valemid, AB Leo, Hälsingborg) and then dried with steril gauze. No anaesthesia was necessary. The area of the specimen was as a rule 10×30 mm. Owing to the different structure of the diseased psoriatic skin, the skin slices obtained from the psoriatic plaques were always about three times thicker than the normal skin slices though the micro adjustment of the dermatome was practically the same

Table 1. *The distribution of the material with respect to clinical data and analyzes made.*

Exp. No.	Case	Biopsy material	Clinical data			O_2 uptake	CO_2 production	Lipids	Lactic acid	Amino acids		Comments
			Age	Duration	Localization					Free	Protein bound	
10	N. B-d	Psoriasis-uninvolved skin	41	20 years	Trunk and elbows	+	+					Dermal section
11	"	Psoriasis-plaque	"	"	"	+	+	+	+			
12	P. H-m	Normal skin	35	—	—	+						
13	R. S-g	Psoriasis-plaque	43	25 years	Trunk, scalp and all extremities	+	+	+	+			
14	"	Psoriasis-uninvolved skin	"	"	" "	+	+		+			
15	C.A. S-g	Normal skin	86	—	—	+	+		+			
17	S. H-n	Psoriasis-plaque	38	23 years	Trunk and all extremities	+	+					
19	"	Psoriasis-uninvolved skin	"	"	"	+	+	+				
20	K. B-k	Normal skin	25	—	—	+	+	+	+	+		
21	J.T. Å-m	Psoriasis-plaque	48	29 years	Trunk and all extremities	+	+	+	+	+	+	
22	"	Psoriasis-uninvolved skin	"	"	"	+	+	+	+			
23	I. E-m	Normal skin	34	—	—	+	+	+	+			
24	L. H-n	Normal skin	39	—	—	+	+	+	+	+		Glucose-C-1 + C-6
26	B. J-n	Psoriasis-plaque	34	12 years	Trunk and all extremities	+	+	+	+	+		Glucose-C-1 + C-6
27	P.O. A-n	Normal skin	20	—	—	+	+	+	+	+		Glucose-C-1 + C-6
28	K. A-n	Psoriasis-plaque	49	23 years	Trunk and scalp	+	+	+	+	+		Glucose-C-1 + C-6
29	B. J-n	Psoriasis-plaque	35	11 years	Trunk and all extremities	+	+	+	+	+	+	
30	E.W. K-n	Psoriasis-plaque	64	35 years	Trunk, scalp and all extremities	+	+	+	+		+	
31A	K.S. A-n	Psoriasis-plaque	54	32 years	Trunk and scalp	+	+		+	+		
31B	"	Psoriasis-uninvolved skin	"	"	"	+	+		+		+	
32	J.V. E-n	Psoriasis-uninvolved skin	61	24 years	Trunk and all extremities	+	+		+	+	+	
33	H. E-n	Psoriasis-plaque	63	45 years	Trunk and all extremities	+	+		+	+	+	
34	"	Psoriasis-uninvolved skin	"	"	"	+	+	+	+	+	+	

35	B. Z-m	Normal skin	20	—	—	+	+	+	+	+	+	
36	M. L-h	Normal skin	23	—	—	+	+	+	+	+	+	
38	Å.W. N-n	Psoriasis-uninvolved skin	45	35 years	Trunk, scalp and all extremities	+	+	+	+	+	+	
40	P. S-n	Normal skin	41	—	—	+	+	+	+	+	+	Glucose-C-1 +C-6
41	S. L-n	Normal skin	32	—	—	+	+	+	+	+	+	Glucose-C-1 +C-6
42	U. S-r	Normal skin	20	—	—	+	+	+	+	+	+	Glucose-C-1 +C-6
43	"	"	"	—	—	+	+		+	+	+	After Kenacort
44	E.A. N-n	Psoriasis-plaque	45	23 years	Trunk, scalp and all extremities	+	+	+	+	+	+	Glucose-C-1 +C-6
45	"	"	"	"	" "	+	+	+	+	+	+	After Kenacort
46	K. S-d	Psoriasis-uninvolved skin	37	15 years	Trunk and all extremities	+	+	+	+	+	+	
47	"	"	"	"	" "	+	+	+	+	+	+	After Kenacort
48	A.E. O-n	Psoriais-plaque	57	25 years	Trunk and all extremities	+	+	+	+	+	+	
50	"	"	"	"	" "	+	+		+	+	+	After Kenacort
51	R.E. J-n	Psoriasis-uninvolved skin	36	15 years	Trunk and all extremities	+	+		+	+	+	
52	"	"	"	"	" "	+	+		+	+	+	After Kenacort
53A	P.O. B-m	Psoriasis-plaque	31	5 years	Trunk and all extremities	+	+		+	+	+	
53B	"	Psoriasis-uninvolved skin	"	"	" "	+	+					
54	"	Psoriasis-plaque	"	"	" "	+	+		+	+	+	After Kenacort
55	B. H-n	Normal skin	33	—	— —	+	+		+	+	+	
56	J.-H. A-g	Normal skin	27	—	— —	+	+		+	+	+	

+ indicates that analyzes were made

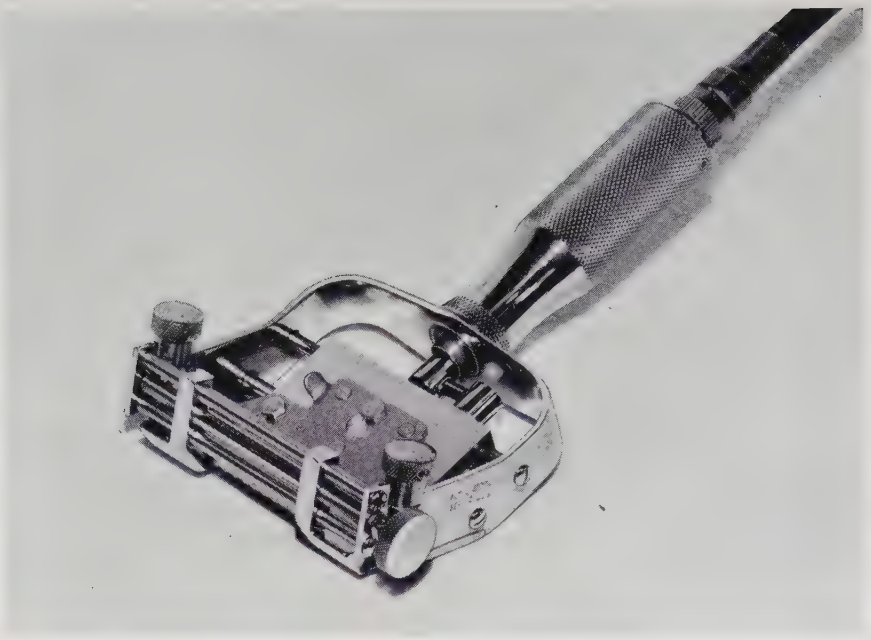

Fig. 1. *The Brown Electro-Dermatome.*

during the entire investigation (Fig. 22). The thickness of the psoriatic and normal skin slices was generally equal to 0.3 and 0.1 mm respectively. These differences seem, however, to be negligible since in no case the limiting thickness for oxygen consumption, which can be calculated for a given tissue (84), was exceeded. In most cases the skin slices thus obtained consisted exclusively of epidermis. For obvious reasons the cut line was different, however, in normal and in psoriatic skin slices, as is demonstrated in Fig. 22. In normal skin or in unaffected skin from psoriatics the cut line generally corresponded to the basal membrane (Fig. 22A—B). In specimens from psoriatic lesions the cut line was on the other hand above the basal membrane. Thus, in many of the cases last mentioned the skin slices consisted entirely of akanthotic epidermal cells covered by parakeratotic cells and a hyperkeratotic horny layer (Fig. 22C). Generally, the total weight of the specimens varied between 30 and 60 mg.

In each case a section of the skin specimen was reserved for microscopic examination. The remaining part of the skin sample was divided into two equal parts for the subsequent analysis, which consequently in most cases was carried out in duplicate.

The number of epithelial cells per surface unit was estimated by direct counting of the cells within an arbitrary cross section of the epithelium in the different skin slices. To facilitate this counting procedure, the microphotographs of the various skin specimens were highly magnified by means of a projector. The exact area, within which the epithelial cells were counted, was, after projection on a white paper, determined by weighing the cut area on a microbalance. The weight of a square dm of this paper had previously been estimated. During this procedure, the borderlines between stratum corneum, epidermis and corium were, furthermore, marked out on another sheet of paper. By weighing the different sections of the magnified microphotographs after they had been accurately cut out, it was possible to determine the percentage of epithelium in all skin samples.

CHAPTER III

BIOCHEMICAL METHODS

The method employed in this study for determining the fate of labeled C^{14}-glucose in human skin slices is based on the original quantitative radiopaperchromatographic technique introduced by *Chain et al.* (6). The principle applied in this method is to separate and identify, by chromatographic procedures, the radioactive metabolites formed after incubation of the biopsy material with radioactive glucose, and to measure their quantitative distribution by means of an automatic scanning device. The complete analytic procedure is schematically illustrated in Fig. 2. This indicates that the experimental part of the investigation can be devided into 4 parts:

1. The incubation procedure
2. The preparation procedure
3. The chromatographic separation procedure
4. The measurement of radioactivity

INCUBATION OF HUMAN SKIN SLICES WITH C^{14}-GLUCOSE

Equipment

Torsion balance (Sauter)
Warburg constant volume respirometer (Braun)

Reagents

Oxygen

Radioactive glucose. Universally labeled glucose, D-Glucose-U-C^{14}, was obtained from the Radiochemical Centre, Amersham, England. D-Glucose-U-C^{14} is prepared by hydrolysis of uniformly labeled sucrose isolated from leaves which have been allowed to photosynthesize in the presence of C^{14}-CO_2 for 10—12 hours. After purification of the hydrolysate by paper chromatography and with ion-exchange resins, the glucose is crystallized from methanol-iso-propanol. It is then dissolved in water, dispensed, freeze-dried and sealed under vacuum.

In a supplementary study skin slices were simultaneously incubated in glucose-1-C^{14} and glucose-6-C^{14} manufactured by the Radiochemical Centre, Amersham. D-Glucose-1-C^{14} is prepared by a Kiliani condensation between D-arabinose and cyanide -C^{14}. D-Glucose-6-C^{14} is prepared by the condensation of cyanide-C^{14} with 1,2-o-iso-propylidene-D-xylo-dialdehydo-pento furanose.

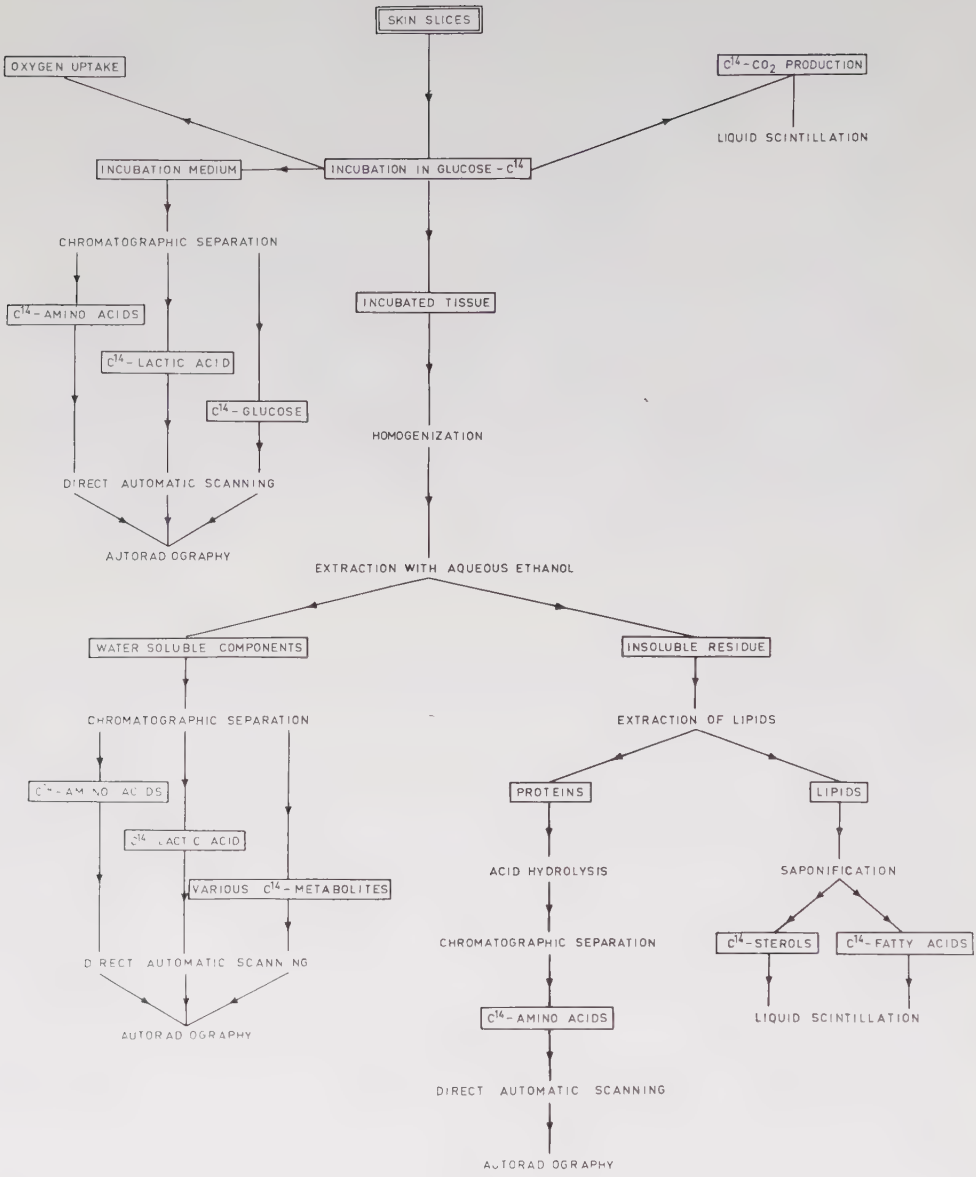

Fig. 2. *Scheme of analytical procedure.*

Incubation medium (*pH 7.3*). The incubation medium had the following basic composition (16):

Na_2HPO_4	0.0175 M
NaCl	0.098 M
KH_2PO_4	0.0004 M
KCl	0.027 M
$MgSO_4$	0.0012 M

Moreover, the incubation medium contained 0.1 per cent glucose with a total radioactivity of 20 μC/ml. To fulfil these conditions the C^{14}-glucose was diluted with inert glucose to the required specific activity.

30 per cent NaOH solution.

Procedure

The rectangular skin specimen was divided into two equal parts, each of which, after weighing, was placed in a Warburg flask of standard size. As soon as possible 1 ml of the incubation medium was pipetted into each flask. Then 0.1 ml of a 30 per cent NaOH solution was carefully pipetted into the center well, containing a rolled and fringed strip of filter paper. The flasks were then connected to the manometers, placed in the constant temperature bath at 37.0° C and gassed with pure oxygen for 5 minutes. The average time from the biopsy to the bath was 10 minutes. After an equilibration period of 15 minutes the manometers were adjusted to the reference point and the flasks were then incubated for exactly 2 hours. The oxygen uptake was generally calculated from manometer readings after 1 and 2 hours. At the beginning of the present study the interval between the manometer readings was 15 minutes. From a series of such investigations it could be proved, however, that the oxygen uptake in these experiments is a linear function, which verifies the unchanged viability of the incubated tissue (Fig. 3). Changes in the atmospheric pressure and in the temperature of the water bath were corrected by using a thermobarometer. This consists of only a Warburg manometer with a flask containing pure water.

After 2 hours the incubation was immediately discontinued by chilling the flasks, while still mounted on the manometers, in a vessel with cold ethanol (—20° C).

The routes of the glucose catabolism in normal and in psoriatic skin slices were studied by comparative, simultaneous incubation of skin slices in glucose-1-C^{14} and glucose-6-C^{14} (glucose, specifically labeled at carbon-1 and carbon-6, respectively). The background to this technique is the assumption that CO_2 derived from glucose-6-C^{14} arises primarily along the Embden-Meyerhof pathway (EMP) whereas CO_2 derived from glucose-1-C^{14} arises along both the EMP and the hexose monophosphate shunt (HMP-shunt). Consequently, the C^{14}-CO_2 production after simultaneous incubation in glucose-1-C^{14} and glucose-6-C^{14} would be the same only if the EMP operated exclusively. Concurrent glucose catabolism along the HMP-shunt would, on the other hand, result in a greater conversion of C^{14} to $C^{14}O_2$ after incubation in glucose-1-C^{14} than in glucose-6-C^{14}. Hence, when the HMP-shunt is operative, the ratio of CO_2 derived from glucose-1-C^{14} to CO_2 from glucose-6-C^{14} $\left(\frac{C_1\text{-}CO_2}{C_6\text{-}CO_2}\right)$ will exceed unity. As recently pointed out by *Katz and Wood* (43) the $\frac{C_1}{C_6}$ ratio for CO_2 has, however, no simple relationship to the relative proportions of the amounts of glucose catabolized along the HMP-shunt and the Embden-Meyerhof pathway. This is mainly due to the possible utilization of the glucose-6-phosphate in other pathways and, moreover, to the recycling of the glucose-6-phosphate via the HMP-shunt. The occurrence of CO_2-fixation indicating that CO_2 can be used in the synthesis of substances required by the organism, must also be emphasized in this connection (69).

The purpose of this study, which included also the determination of C^{14}-amino acids and C^{14}-lipids, was primarily to verify the recent demonstration (44) of high activity of the HMP-shunt in human skin. Moreover, it seemed to be of particular interest to compare the activity of the HMP-shunt in normal and psoriatic skin slices.

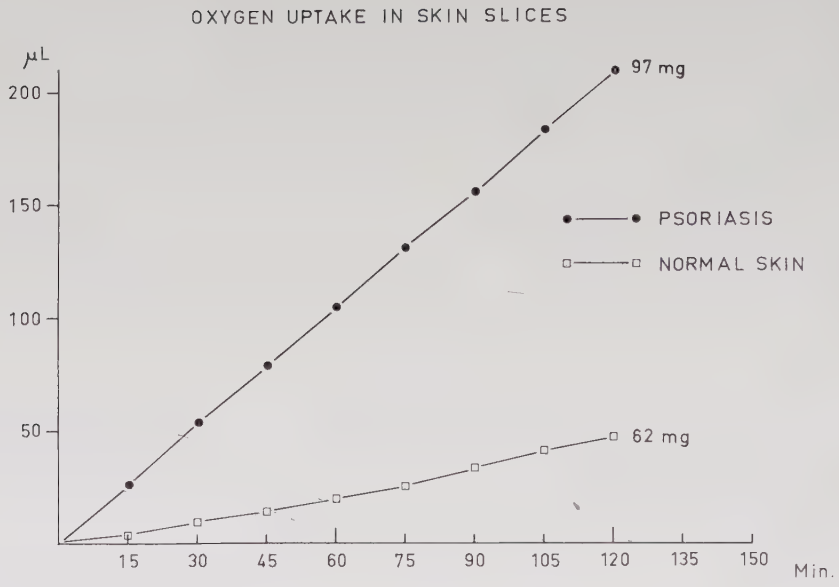

Fig. 3. *Oxygen uptake in normal and in psoriatic skin slices.*

In a supplementary investigation the effect of Triamcinolone therapy on the psoriatic skin process was also studied using universally as well as specifically labeled glucose. The patients, included in this study, were given orally 4 mg of Kenacort four times daily during 5 days. Skin specimens from the same gluteal area were taken before and immediately after the steroid treatment.

Repeated bacteriologic examinations of the stored C^{14}-glucose substrate and the incubation medium were made after the completion of the preparations using Na-thioglykolate-ascites-broth (Bacteriologic department, Södersjukhuset, Stockholm). These examinations, however, did not reveal any signs of bacterial growth except, in some cases, after the enrichment of the incubation medium on hoarse blood agar plate. Hence, the metabolic changes in human skin slices which will be subsequently discussed, cannot be due to any metabolic action of the remaining skin bacterias.

PREPARATION OF SAMPLE

Equipment

Pyrex glass homogenizers 5 ml (AB Termo-glas, Göteborg)
Pasteur pipettes
Centrifuge (AB Wifug, Stockholm)
Rotating vacuum evaporater (manufactured by AB Stålprodukter, Uppsala)
Pyrex glass tubes for protein hydrolysis
Micro pipettes (AB Wernerglas) 0.2 and 0.3 ml
Vacuum desiccator (AB Kebo, Stockholm)
Glass-stoppered separation funnels 50 ml
Freezer (—20° C)
Thermostatic heater (AB Kifa, Stockholm)

Reagents ("Reagent" quality)

Isotonic sodium chloride solution
Hydrochloric acid 6 N
Phosphorus pentoxide
Ethanol 60 %
Absolute ethanol-acetone 1 :1
Alkaline ethanol. This was prepared by dissolving 4 g potassium hydroxide in 5 ml of water in a 100 ml volumetric flask, which was filled up to the mark with absolute ethanol.
Petroleum ether b.p. 30—50° C
Alkaline thymol blue solution:

Absolute ethanol	250 ml
Water	220 ml
1-N NaOH	30
Thymol blue to blueish colour	

Ethanol 50 %, coloured with crystal violet
Toluene

Procedure

Immediately after the incubation, the filter-paper strip in the center well was transferred to a test tube containing 10 m of water. Subsequently, the center well was washed several times by the aid of a Pasteur pipette. An aliquot of this aqueous solution (0.2 ml) was pipetted into a counting vial, to which 10 ml of alcoholic PPO-toluene was then added. The C^{14}-CO_2 production was calculated from the registered radioactivity of the aqueous solution of sodium C^{14}-carbonate, and expressed as μg glucose converted into carbon dioxide per 100 mg of tissue (wet wt.)after 2 hours incubation.

The incubation medium was pipetted into small test tubes while the tissue, after thorough washing in isotonic sodium chloride solution, was transferred to a homogenizer. The tissue was then homogenized with the homogenizer chilled in cold ethanol (—20° C), extracted with 60 per cent ethanol and transferred quantitatively to a centrifuge tube. After centrifugation, the supernatant fluid was evaporated to dryness in a rotating vacuum evaporator at 35° C. The evaporation residue was then dissolved in 0.3 ml of water, which was aspirated in an Agla micrometer syringe for chromatographic separation.

The insoluble residue was extracted with 5 ml of ethanol-acetone. After centrifugation the supernatant was preserved for lipid analysis. The sediment was suspended in 1 ml of 6-N HCl, which was transferred quantitatively to a small ampouleshaped Pyrex glass tube by means of a Pasteur pipette. For hydrolysis of the proteins, the sealed glass tube was then placed in a thermostatic heater at 110° C for 24 hours. The hydrolysate was evaporated to dryness in a vacuum desiccator containing phosphorus pentoxide. The residue was extracted with 60 per cent ethanol, which was evaporated to dryness in the vacuum evaporator. This new residue was redissolved in 0.3 ml of water and aspirated in a micrometer syringe for chromatographic separation.

The lipids were saponified and separated into sterols and fatty acids by means of a technique earlier described by Borgström (10).

The first step in this procedure was to evaporate the ethanol-acetone extract to dryness in a heater at 100° C. The residue was saponified at room temperature in 5 ml of alkaline ethanol for about one hour. After filtering into a 50 ml separation funnel, this was supplied with 20 ml of petroleum ether, 10 ml of the alkaline thymol blue solution and 10 ml of water. The mixture was shaken vigorously for some minutes. After separation, the lower phase, containing the water-soluble saponified lipids, was transferred to the next separation funnel, containing 20 ml of petroleum ether, acidified to a yellow colour with 1 N HCl and again shaken. During this procedure, the liberated free fatty acids were dissolved in the upper petroleum ether phase, which was afterwards washed at least 3 times with 50 per cent ethanol. The upper phase in the first funnel, containing the unsaponified sterols, was

shaken once more with 10 ml of alkaline thymol blue solution, which was then made to pass through the next funnel with renewed acidification etc. The sterol phase was also washed 3 times with 50 per cent ethanol. Both phases were evaporated to dryness in a vacuum evaporator and redissolved in 10 ml of toluene. 4 ml of the toluene solution was pipetted into a counting vial, containing 8 ml of PPO-toluene.

CHROMATOGRAPHIC PROCEDURES

Equipment

Chromatographic chambers for descending development of one-and two-dimensional chromatograms. The chambers for development of one-dimensional chromatograms had the following dimensions: height 60 cm, length 30 cm and width 20 cm. They were provided with 2 glass troughs, permitting 4 papers (23 × 50 cm) to be run simultaneously. The chamber for two-dimensional chromatography was an original Shandon chamber. It was provided also with 2 glass troughs and could take 4 papers (45 × 50 cm) simultaneously.
Whatman paper No. 1
Agla micrometer syringes
Electrical hair dryers

Reagents

Solvent systems:

1. sec-Butanol:formic acid:water = 75:15:15 v/v
2. Phenol:water:ammonia = 80 g:20 ml:0.5 ml
3. tert-Butanol:picric acid:water = 80 ml:4 g:20 ml
4. n-Butanol:acetic acid:water = 40:11:25 v/v

All solvents were freshly prepared immediately before using. The ammonia solution (d = 0.910) was stored at —20° C. Solvent No. 2 was prepared from freshly distilled phenol. All reagents were of "Reagent" quality.
Reference solutions. The amino acids were used in 0.1 per cent aqueous solution 10 µl of which was sufficient to produce a distinct spot on the chromatogram after spraying with ninhydrin solution.
Ninhydrin solution. 0.25 g of ninhydrin was dissolved in 100 ml of acetone.
Ammonia solution (d = 0.910)

Procedure

The chromatographic procedures were carried out in a dark room at a temperature of 22 ± 2° C.

Application of samples

The solution to be analyzed (incubation medium, extract of the tissue or hydrolysate) was applied to the start point in small portions not exceeding 2 µl by an Agla micrometer syringe. The spot was dried by air from an ordinary hair dryer mounted below the start point of the horizontally suspended filter paper. Cold air was used except for the lactic-acid chromatograms.

Chromatographic technique

For two-dimensional descending chromatography solvent No. 1 and solvent No. 2 were used. This technique was employed for the separation and identification of both the free and protein-bound amino acids (Fig. 6 & 10). One-dimensional descending chromatography was employed for determining the C^{14}-glucose level in the incubation medium (Solvent No. 3—Fig. 8) and for estimating the amount of C^{14}-lactic acid in the incubation medium and the extract of the tissue (Solvent No. 4 —

Fig. 9). No pre-equilibration of the papers was necessary. After the run, the papers were dried at room temperature. The lactic-acid chromatograms were previously hung, however, for about 20 minutes in a separate chamber containing ammonia solution in order to convert the lactic acid into ammonium lactate, which is a more stabile compound.

The amount of the test material applied to each sheet of paper varied between 10 and 50 µl. For two-dimensional chromatography 50 µl was used. One-dimensional chromatography was carried out with either 10 µl (incubation medium) or 20 µl (extract of the tissue).

Identification of spots

The reference solutions, containing different known amino acids, were applied by the above-mentioned technique for two-dimensional chromatography (solvents No. 1 and 2). After the papers had been dried, ninhydrin solution was applied by spraying. The chromatograms were then heated at about 100° C for 5 minutes in order to develope the coloured spots. The Rf values of the different amino acids were calculated and compared with the values obtained from the autoradiochromatograms. In this way it was possible to identify practically all the C^{14}-metabolites (Fig. 12 and 13).

MEASUREMENT OF RADIOACTIVITY

A. AUTOMATIC SCANNING OF RADIOPAPERCHROMATOGRAMS

The radiochromatograms were scanned by means of an electronic automatic scanning device, developed by *Chain et al.* (3), improved by *Frank* (24), and manufactured by the SANSAR Elettronica Per Fisica Nucleare, Milano (Fig. 4).

In this apparatus the radiochromatogram is vertically placed between two mica window GM-tubes which count the β-particles from an area, one square cm, for a time period which can be set to any integral number of seconds between 10 and 100. The chromatogram is taped to a blank sheet of paper inserted into the carriage of an electric typewriter, the number keys of which are actuated by a series of solenoids through an electronic read-out. After finishing the counting for the set time period the typewriter prints the counts recorded by the GM-tubes, simultaneously moving the chromatogram to the next area, which is to be scanned. Since the apperture in front of the GM-tubes is exactly adjusted with regard to the movements of the typewriter carriage during the printing and line change procedure the entire chromatogram will be scanned without any overlapping.

To reduce the total scanning time, which for a 2-dimensional radiochromatogram, scanned at 60 seconds intervals, would take about 15 hours, the apparatus is fitted with a scanning time self-setting device. This automatically reduces the counting time for those areas of the chromatogram, where the activity is beyond a certain set level, considered as the background activity. In this way, the time used for scanning of the chromatogram can be reduced to about 5 hours without increasing the statistical error of the recorded counts within the radioactive spots.

To ensure a high degree of accuracy, all chromatograms in this study were scanned at 60 seconds intervals. The one-dimensional chromatograms for measurement of the lactic acid production were scanned immediately after they had been dried. The remaining radiochromatograms were scanned within 2—3 days after incubation.

Calculations

The total radioactivity corresponding to a spot on the radiochromatogram was calculated after deduction of the background activity by adding the recorded counts within the area in view (Fig. 5). Since the total amount of glucose in the incubation medium was known, it was possible to estimate the number of recorded counts that corresponded to 1 mg glucose, by chromatographic development of an aliquot of the incubation medium, with subsequent scanning. Consequently, the radioactive

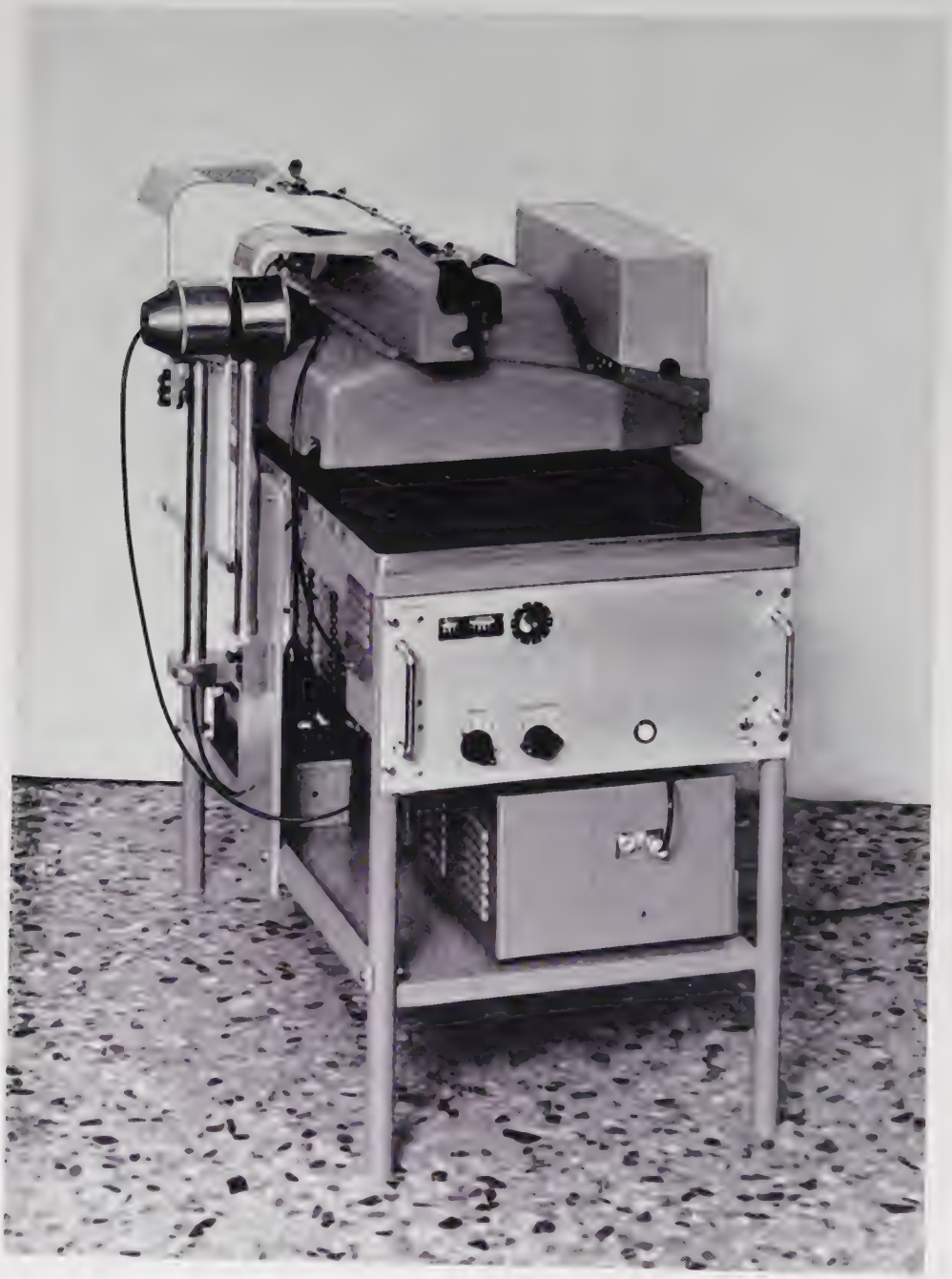

Fig. 4. *Automatic scanner for the quantitative evaluation of two-dimensional radiochromatograms.*

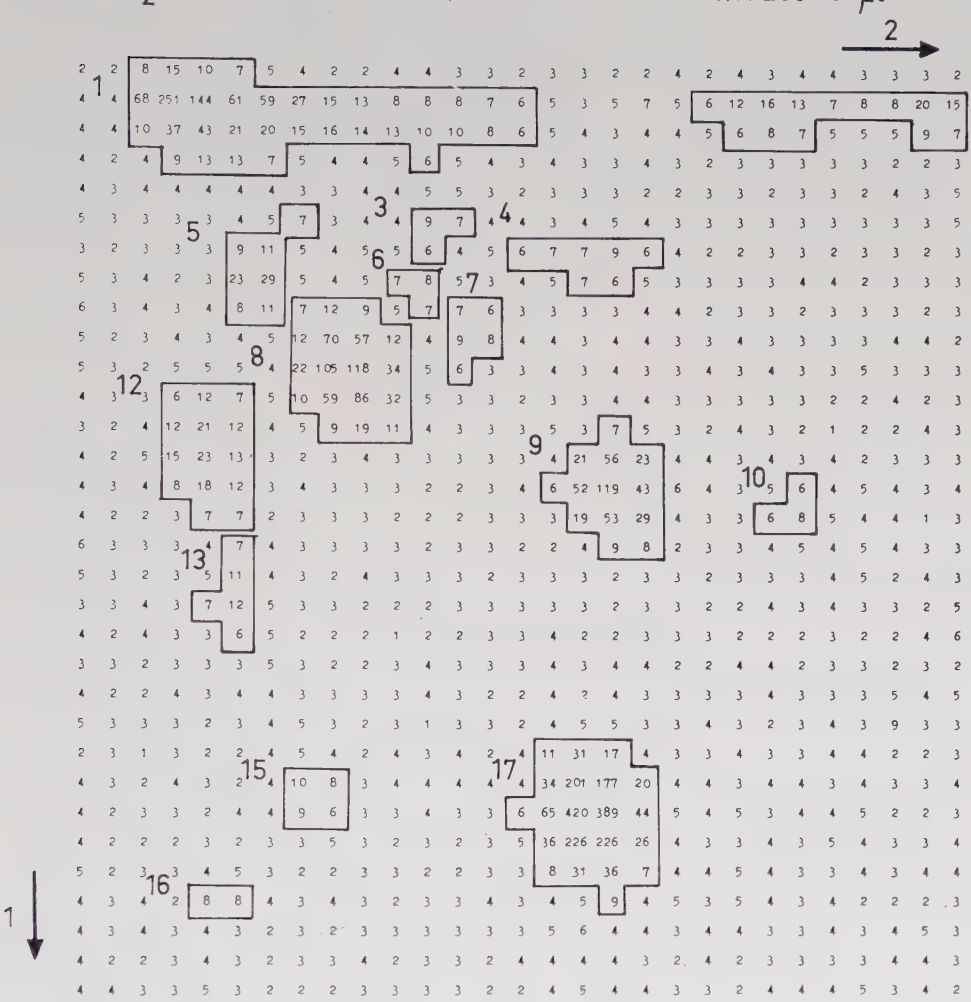

Fig. 5. *Direct automatic scanning of a radiochromatogram of the tissue extract after incubation of psoriatic skin with C^{14}-glucose.*
Two-dimensional chromatogram developed in solvent No. 1 (↓) and solvent No. 2 (→). For identification of the different spots see Table 5.

metabolites derived from C^{14}-glucose, which were separated by chromatographic procedures and scanned quantitatively, were expressed *as µg glucose converted.* Moreover, all results presented in this study were calculated per 100 mg of tissue (wet wt.) after 2 hours incubation.

B. LIQUID SCINTILLATION COUNTING

Equipment

Tri Carb Liquid Scintillation Spectrometer (Packard Instrument Company Inc., Illinois). The principal components of the counting system are the detector, the freezer and the electronic unit.

The detector consists of two photo-multiplier tubes, mounted in-line, and a reflecting cell.

The freezer contains the detector, the preamplifiers and the sample chamber. This arrangement provides the required low temperature for low background operation.

The electronic unit includes two linear amplifiers, a dual channel coincidence amplifier and two scalers.

The light pulses developed in the scintillator solution are transmitted to the photomultiplier tubes, where they are converted into electrical impulses. These impulses, amplified by preamplifiers and linear amplifiers, are fed to discriminators, which reject the smaller noise pulses. Pulses which pass the discriminators are applied to a coincidence gate which rejects non-coincident noise pulses. A second discriminator (blanking gate) rejects large noise pulses, thus passing to the scaler only the true counts from the sample plus minimal amount of background counts.

Reagents

PPO-toluene. 3.2 g PPO (2,5-diphenyloxazole) dissolved in 1,000 ml of toluene of "Reagent" quality.

Alcoholic PPO-toluene. 700 ml of PPO-toluene were added to 300 ml of absolute ethanol.

Procedure

This technique was mainly employed for determining the C^{14}-CO_2 and C^{14}-lipid production. Since, in recent years, liquid scintillation counting has become the method of choice for the measurement of soft beta-activity, this technique was initially employed in this investigation also for counting the radiochromatograms. To facilitate this counting procedure, only those parts of the paperchromatograms were measured, which corresponded to the different radioactive spots detected by autoradiography. These areas were cut into 15×15 mm sections. These were inserted in pairs in standard counting vials containing 0.2 ml of water and 15 ml of alcoholic PPO-toluene. The vials were then counted in a Tri Carb liquid scintillation spectrometer with the proper voltage settings and optimal window widths for the radioisotope in question.

The counting efficiency with this technique seemed to be fairly high and in agreement with the data recently presented by *Wang and Jones* (85). In view of the very prolonged procedure and the disadvantage of the long time interval between the incubation and the counting, it was necessary, however, to restrict the liquid scintillation counting to the C^{14}-CO_2 and the C^{14}-lipid production. Hence, all radiochromatograms in the later part of the investigation were scanned directly by means of the technique previously described.

For estimating the C^{14}-CO_2 production, an aliquot (0.2 ml) of the sodium C^{14}-carbonate solution was pipetted into a counting vial, to which 10 ml of alcoholic PPO-toluene was added.

The counting of the two C^{14}-lipid fractions, sterols and fatty acids, was performed by transferring 4 ml of the sample into a vial, containing 8 ml of PPO-toluene.

Calculations

The total radioactivity of each vial was estimated by deduction of the background activity, which was measured by counting a blank vial.

The liquid scintillation-counting value of 1 μg glucose was calculated by counting an aliquot of the incubation medium. After correction for the weight factor, the C^{14}-CO_2 and C^{14}-lipid formation was computed and expressed *as μg glucose converted* per 100 mg of tissue after 2 hours incubation.

C. AUTORADIOGRAPHIC PROCEDURE

Equipment

Dark-room furnished with developing and fixing bath.
Black, lightproof boxes 30×40 cm.
X-ray cassettes 30×40 cm.
Kodirex X-ray film 30×40 (Kodak Ltd.)

Reagents

Developing bath: Gevaert G 230
Fixing bath: Gevaert G 305 A

Procedure

The autoradiographic technique was used to enable the identification of the radioactive metabolites, formed by C^{14}-glucose, which had previously been separated by chromatographic procedures.

Prior to the exposure, the start and front lines of the chromatograms were filled up using radioactive black India ink. The radiochromatograms were firmly attached to the emulsion of the X-ray films, either by using X-ray cassettes or by pressing between two glass plates, which were stored in lightproof boxes. In each case the exposure time was 14 days. The films were developed and fixed by a scrupulously standardized technique regarding temperature, time and freshness of the developing solutions.

The different spots on the autoradiochromatograms were identified by comparing the R_f values with the values obtained by chromatographic development of known substances (Fig. 12 and 13).

5. STATISTICAL METHODS

As earlier pointed out, all skin samples were analysed in duplicate. The standard deviation (S) and the variance (S^2) were calculated from the following formula, where x represents the individual value (=average of two determinations) and N the total number of individual values:

$$S^2 = \frac{1}{N-1}\left(\Sigma(x^2) - \frac{(\Sigma x)^2}{N}\right)$$

The mean values were given ± s.e.m. (s.e.m. = standard error of the mean). The significance of the difference between two means, $\bar{x}_1$ and $\bar{x}_2$, was tested by dividing the difference $(\bar{x}_1 - \bar{x}_2)$ by its standard error, $S_P \cdot \sqrt{\frac{N_1+N_2}{N_1 \cdot N_2}}$, where S_P is the common standard deviation. S_P was calculated as $\sqrt{\frac{S_1^2(N_1-1)+S_2^2(N_2-1)}{N_1+N_2-2}}$ If this test gave a higher value than corresponding t-value at a significance level of 1 %, the difference was regarded as statistically significant.

CHAPTER IV

RESULTS

OXYGEN UPTAKE

As previously pointed out, it is possible to show by repeated manometer readings at 15 minutes intervals, that the oxygen uptake is a linear function during the entire incubation, which demonstrates the unchanged viability of the incubated tissue (Fig. 3). Table 2 shows the oxygen consumption, by normal and psoriatic skin slices, calculated per 100 mg of tissue (wet wt.) after 2 hours incubation. Primarily, this indicates that the oxygen uptake is conspiciously higher in the psoriatic plaque than in either the normal skin or the uninvolved skin from psoriatics. Furthermore, the oxygen consumption is higher in the uninvolved skin from psoriatics than in the normal skin; this difference is highly statistically significant ($P < 0.001$).

In one experiment, case 10, where the cut line happened to penetrate deep into the dermis (Fig. 22 D), the incubated tissue was composed of nearly equal parts of epidermis and dermis. The oxygen uptake of this specimen, a psoriatic's uninvolved skin, was only 57 µl. This finding is in agreement with the previous observation that the respiration is significantly higher in the epidermis alone than in the entire skin (47).

Table 2. *Oxygen uptake in normal and psoriatic skin slices.*

Results expressed as µl and µM of oxygen per 100 mg of tissue (wet wt.) after 2 hours incubation at 37° C in pure O_2. The values are given as $\bar{x} \pm$ s.e.m. Number of experiments in parenthesis.

Group	Oxygen uptake	
	µl	µM
I. Normal skin (13)	100.0 ± 4.6	4.5 ± 0.2
II. Psoriasis-uninvolved skin (10)	130.2 ± 5.5	5.8 ± 0.2
III. Psoriasis-plaque (13)	221.2 ± 12.1	9.9 ± 0.5

t-Analysis of differences in oxygen uptake between group I and II

I—II $t = 4.24^{***}$, $P < 0.001$

CARBON DIOXIDE PRODUCTION

Table 3 shows the C^{14}-CO_2 production in the different skin samples. Obviously, the production of carbon dioxide is much higher in the diseased psoriatic skin than in the normal skin or in the uninvolved skin of psoriatics; these findings correspond to the oxygen consumption of the different tissues, reported above. Analogously, the mean CO_2 production is higher in the uninvolved skin of psoriatics than in normal skin. The difference is almost statistically significant ($P<0.02$).

Since it has not been possible to determine, with the technique employed in this study, the total amount of carbon dioxide produced by the tissue during incubation, the reported RQ-values are, of course, only fictive. In comparison with the true RQ-values for the skin, previously reported, (65) they nevertheless indicate that most of the carbon dioxide, absorbed by the NaOH solution in the center well, emanates from the metabolism of endogenous glucose in the incubated skin slices. Analogously, the higher RQ-values in both the psoriatic groups compared with the normal skin indicate an enhanced utilization of labeled, exogenous glucose, i.e. an increased uptake of glucose, in psoriatic skin slices.

The CO_2-production in the epidermal-dermal skin slice (case 10) was equivalent to 7 μg of glucose in the incubation medium, which reflects the low respiration in the dermis.

Table 10 illustrates, among other data, the carbon dioxide production by simultaneous incubation of normal and psoriatic skin slices in glucose-1-C^{14} and glucose-6-C^{14}. The significant increase in C_1-CO_2 production in all samples warrants the statement that the HMP-shunt operates by the interconversion of glucose in both normal and psoriatic skin.

Table 3. *C^{14}-carbon dioxide production in normal and psoriatic skin slices.*

Results expressed as μg of glucose converted into CO_2 per 100 mg of tissue (wet wt.) after 2 hours incubation at 37° C in pure O_2. Glucose concentration 0.1 %. Total radioactivity 20 μC per ml phosphate medium, pH 7.3. The values are given as $\bar{x} \pm$ s.e.m. Number of experiments in parenthesis.

Group	C^{14}-CO_2 production		μM C^{14}-CO_2 / μM O_2
	μg of glucose	μM	
I. Normal skin (7)	24.7 ± 3.5	0.8 ± 0.1	0.18
II. Psoriasis-uninvolved skin (10)	42.1 ± 4.5	1.4 ± 0.1	0.24
III. Psoriasis-plaque (10)	90.8 ± 6.6	3.0 ± 0.2	0.30

t-Analysis of differences in CO_2 production between the groups

I—II $t = 2.74^{\star}$, $P < 0.02$
I—III $t = 24.60^{\star\star\star}$, $P < 0.001$
II—III $t = 6.12^{\star\star\star}$, $P < 0.001$

Table 4. *Lactic acid formation in normal and psoriatic skin slices.*

Results expressed as μg of glucose converted into lactic acid per 100 mg of tissue (wet wt.) after 2 hours incubation at 37° C in pure O_2. Glucose concentration 0.1 %. Total radioactivity 20 μC per ml phosphate medium, pH 7.3. The values are given as $\bar{x} \pm$ s.e.m. Number of experiments in parenthesis.

Group	Lactic acid formation (in per cent of total uptake of glucose)
I. Normal skin (7)	86.7 ± 1.7
II. Psoriasis-uninvolved skin (8)	90.7 ± 4.2
III. Psoriasis-plaque (9)	73.8 ± 2.3

t-Analysis of differences in lactic acid formation between the groups

I—II $t = 0.83$, $P < 0.5$
I—III $t = 4.29^{***}$, $P < 0.001$
II—III $t = 3.64^{**}$, $P < 0.01$

LACTIC ACID FORMATION

The amount of lactic acid derived from glucose was determined by scanning the radiochromatograms run in solvent No. 4 (Fig. 9). Since as much as 90—95 per cent of the lactic acid produced by the tissue during incubation was found in the incubation medium, it was necessary, after chromatographic separation, to estimate the total lactic acid formation by adding the activity of the aqueous ethanolic extract of the tissue to the activity of the incubation medium. From Table 4 the conclusions may primarily be drawn that the major part of the assimilated glucose was converted into lactic acid. Thus, the lactic acid produced, generally accounted for 75—90 per cent of the total uptake of glucose.

Table 4 demonstrates, moreover, that the lactic acid formation, expressed in per cent of the total uptake of glucose, was significantly lower in psoriatic plaques than in normal skin or uninvolved psoriatic skin ($P < 0.001$).

AMINO ACID FORMATION

The qualitative distribution pattern of the free amino acids derived from glucose in normal and psoriatic skin is illustrated in Fig. 6 and 7. The identification of the different C^{14}-metabolites is evident from Table 5. Of the free amino acids formed from glucose in human skin slices it is apparent that glutamic acid, alanine and aspartic acid are definitely dominant, indicating, primarily, the importance of the transamination reactions in the biology of the skin.

Table 5. *C^{14}-metabolites derived from C^{14}-glucose and separated by 2-dimensional chromatography.*

SPOT No.	C^{14}-Metabolite	R_f value[1]
1	Glycogen and oligosaccharides	(start)
2	Maltose	—
3	Glucose	—
4	Glutamine	0.2 : 0.6
5	Aspartic acid	0.2 : 0.1
6	Serine	0.2 : 0.3
7	Glycine	0.3 : 0.4
8	Glutamic acid	0.3 : 0.2
9	Alanine	0.4 : 0.6
10	GABA	0.4 : 0.8
11	Proline	0.4 : 0.9
12	Citric acid	—
13	Maleic acid	—
14	α-Ketoglutaric acid	—
15	Succinic acid	—
16	Pyruvic acid	—
17	Lactic acid	—
18	Leucine-isoleucine	0.7 : 0.8

[1] Solvent 1: solvent 2 (Reference solution).

In this connection, it should be emphasized that gamma-aminobutyric acid (GABA) is found in most chromatograms of the aqueous ethanolic extract and the incubation medium (Fig. 6, 7, 20). This amino acid, a derivative from glutamic acid, which commonly has been regarded as specific for brain tissue (56) has not previously been recorded in the skin, either animal or human.

The comparison between the different two-dimensional autoradiochromatograms has clearly revealed that there are mainly quantitative differences in the metabolic pattern of the various skin samples (Fig. 6, 7, 16 and 17). *Hence, no C^{14}-intermediate, specific for either diseased or uninvolved skin in psoriasis was registered.*

The quantitative evaluation of the two-dimensional radiochromatograms with regard to the conversion of glucose into free amino acids has previously, as a rule, been restricted to investigations of the aqueous ethanolic extract of the incubated tissue (15, 17). This was done because the amount of free amino acids found in the substrate after incubation of for example brain and diaphragm tissue was negligible. After incubation of human skin slices, however, considerable quantities of labeled glutamic acid and alanine were found in the substrate (Table 6 A). Further, notable amounts of GABA were generally found in the incubation medium while, on the other hand, the occurrence of labeled aspartic acid was limited to the tissue extracts. These findings show that the free amino acids converted from the C^{14}-glucose during incubation of skin slices to a varying degree enter the incubation medium. This might be due to the excretory nature of the epidermal cells. Consequently, by quantitative evaluation of

Table 6 A. *Formation of free C^{14}-amino acids in psoriatic skin slices after incubation during different time intervals.*

Results expressed as μg of glucose converted into amino acids per 100 mg of tissue (wet wt.).

Incubation time	Aspartic acid		Glutamic acid		Alanine		GABA	
	Extract	Substrate	Extract	Substrate	Extract	Substrate	Extract	Substrate
15 min.	0.5	—	4.4	7.3	1.0	1.0	—	1.0
75 min.	2.2	—	11.8	13.9	1.0	4.6	—	3.0
135 min.	3.5	—	12.6	15.5	2.7	11.9	—	3.6

Explanation: Extract = Aqueous ethanolic extract of the incubated tissue
Substrate = Incubation medium

Table 6 B. *Formation of protein-bound C^{14}-amino acids in psoriatic skin slices after incubation during different time intervals.*

Incubation time	Aspartic acid	Glutamic acid	Alanine	Leucine-isoleucine
15 min.	—	0.3	0.4	—
75 min.	1.8	6.1	3.2	1.0
135 min.	6.6	16.8	10.1	4.4

the free amino acid formation in skin slices it is necessary to determine the amino acid content both in the incubation medium and in the tissue extract. Finally, the data presented in Table 6 A indicate that the production of free amino acids from glucose is obviously not a linear function.

In this connection it should be emphasized that the degree of sensitivity of the automatic scanning device, used in this study, is not sufficiently high to ensure the recording of very faint spots. Actually, the autoradiographic technique employed in the present work has been found to be more sensitive than direct scanning and consequently more reliable for the qualitative evaluation of the radiopaperchromatograms. This assertion can be confirmed e.g. by comparing one of the typed scanning papers with the corresponding autoradiochromatogram (Fig. 18 B and 21). As shown in these figures the direct scanning of the radiochromatogram in question has failed to reveal, inter alia, any formation of GABA, though a distinct GABA-spot can be recognized on the autoradiochromatogram.

Table 7. *Formation of free amino acids in normal and psoriatic skin slices.*

Results expressed as μg of glucose converted into free amino acids per 100 mg of tissue (wet wt.) after 2 hours incubation at 37° C in pure O_2. Glucose concentration 0.1 %. Total radioactivity 20 μC per ml phosphate medium, pH 7.3. The values are given as $\bar{x} \pm$ s.e.m. Number of experiments in parenthesis.

Group	Free amino acids		
	Aspartic acid	Glutamic acid	Alanine
I. Normal skin (5)	1.8 ± 0.6	25.1 ± 2.9	25.3 ± 1.6
II. Psoriasis-uninvolved skin (6)	2.5 ± 0.9	28.3 ± 0.7	22.3 ± 2.5
III. Psoriasis-plaque (7)	2.2 ± 0.5	17.6 ± 1.8	15.3 ± 2.9

The quantitative distribution pattern of the most important free amino acids in normal and psoriatic skin slices is demonstrated in Table 7. As will be seen here, there are no major differences between the three types of skin slices with regard to the formation of aspartic acid, glutamic acid and alanine.

PROTEIN FORMATION

The insoluble residue, obtained after incubation of human skin slices with subsequent extraction, was found to contain a significant radioactivity due to the biosynthesis of C^{14}-proteins and C^{14}-lipids.

Hydrolysis of the C^{14}-proteins invariably yielded in each skin sample a characteristic pattern of 4 amino acids, *aspartic acid, glutamic acid, alanine and leucine-isoleucine* (Fig. 10, 11 and 12). As is demonstrated already by these autoradiochromatograms, the protein production is abundantly increased in the psoriatic plaque compared with normal skin or uninvolved skin in psoriatics. This statement is furthermore confirmed by the quantitative evaluation of the corresponding radiochromatograms. The results of this investigation are summarized in Table 8. The data presented here show that the formation of protein-bound amino acids is significantly higher in the psoriatic plaque than in normal skin or in uninvolved skin from psoriatics ($P<0.001$). Moreover, it seems that the protein production is somewhat higher in uninvolved skin from psoriatics than in normal skin. The difference is statistically significant for $P<0.1$. It should also be emphasized that the amount of glucose converted into glutamic acid corresponds to nearly 50 per cent of the total amino acid formation.

Incubation of skin slices, taken simultaneously from the same psoriatic patient, during different time intervals revealed that the formation of protein-bound amino acids is an approximately linear function due to a successive building up of the C^{14}-amino acids into proteins (Table 6 B and Fig. 19 A—C).

Table 8. *Formation of protein-bound amino acids in normal and psoriatic skin slices.*

Results expressed as μg of glucose converted into protein-bound amino acids per 100 mg of tissue (wet wt.) after 2 hours incubation at 37° C in pure O_2. Glucose concentration 0.1 %. Total radioactivity 20 μC per ml phosphate medium, pH 7.3. The values are given as $\bar{x} \pm$ s.e.m. Number of experiments in parenthesis.

Group	Protein-bound amino acids				
	Aspartic acid	Glutamic acid	Alanine	Leucine-isoleucine	*Total*
I. Normal skin (5)	—[1]	1.3 ± 0.4	2.4 ± 0.8	1.1 ± 0.4	5.0 ± 1.4
II. Psoriasis-uninvolved skin (7)	1.3 ± 0.4	4.6 ± 0.9	4.1 ± 0.7	0.7 ± 0.2	10.9 ± 2.2
III. Psoriasis-plaque (7)	5.6 ± 0.5	14.2 ± 1.4	7.5 ± 1.0	2.0 ± 0.7	29.3 ± 3.2

t-Analysis of differences in total amounts of protein-bound amino acids between the groups

I—II $t = 2.03^{\star}$, $P < 0.1$
I—III $t = 5.90^{\star\star\star}$, $P < 0.001$
II—III $t = 4.71^{\star\star\star}$, $P < 0.001$

[1] Aspartic acid was found only in trace amounts in two cases in this group.

Fig. 14 and 15 show the total amount of hydrolyzed amino acids in the skin, which were separated by two-dimensional chromatography and detected by ninhydrinspraying. As some spots are not visualized in the autoradiochromatograms, they consequently represent amino acids which have not been identified in the present study. Apparently, there are not even any qualitative differences between diseased and uninvolved skin in psoriasis as regards the distribution of the inert hydrolyzed amino acids.

LIPID FORMATION

In view of the high sensivity of the liquid scintillation spectrometer, it has been possible in the present work to measure the small amounts of C^{14}-lipids, derived from C^{14}-glucose, which could be extracted from the insoluble residue after incubation of normal and of psoriatic skin slices. The results of this study are summarized in Table 9. Primarily, this shows that approximately equally small amounts (0.3—0.4 μg of glucose converted) of both sterols and fatty acids were produced by incubation of normal skin and of uninvolved skin from psoriatics. On the other hand, the C^{14}-lipid formation in the psoriatic plaque was generally about 6 times greater as regards both sterols and fatty acids. Moreover, Table 10 shows that the same amounts of C^{14}-sterols and C^{14}-fatty acids were produced by simultaneous incubation with glucose-1-C^{14} and glucose-6-C^{14} of normal and of psoriatic skin slices.

Table 9. *Lipid formation in normal and psoriatic skin slices.*

Results expressed as μg of glucose converted into lipids per 100 mg of tissue (wet wt.) after 2 hours incubation at 37° C in pure O_2. Glucose concentration 0.1 %. Total radioactivity 20 μC per ml phosphate medium, pH 7.3. The values are given as $\bar{x} \pm$ s.e.m. Number of experiments in parenthesis.

Group	Lipid formation	
	Sterols	Fatty acids
I. Normal skin (9)	0.3 ± 0.06	0.3 ± 0.05
II. Psoriasis-uninvolved skin (5)	0.4 ± 0.11	0.4 ± 0.10
III. Psoriasis-plaque (9)	2.4 ± 0.53	1.8 ± 0.3

MISCELLANEOUS RESULTS

The results of incubation of normal and psoriatic skin slices with specifically labeled glucose are summarized in Table 10. This primarily shows that there is a significantly greater CO_2 formation from glucose-1-C^{14} than from glucose-6-C^{14} in both normal and psoriatic skin slices, suggesting the operation of non-triose pathways, probably the direct oxidative pathway (HMP-shunt). The formation of lipids, amino acids and lactic acid is, on the other hand, about the same from both types of labeled glucose in all skin samples indicating that most of the glucose appears to be metabolized along the glycolytic and citric acid pathway (EMP). Table 10 also demonstrates the greatly increased carbon dioxide and lipid production in psoriatic plaques compared to normal skin. No difference between these two groups of skin samples could, however, be established as regards the HMP-shunt activity.

Table 11 illustrates the effects of systemical steroid treatment on the utilization of labeled glucose in psoriatic skin slices. The experiments conducted with specifically labeled glucose clearly indicate that Triamcinolone (Kenacort) does not stimulate the HMP-shunt activity. The $\frac{C_1}{C_6}$ ratio for CO_2 is thus the same before and after the steroid treatment. The most striking feature of this investigation is the pronounced influence of Kenacort on respiration both with regard to oxygen uptake and to carbon dioxide formation. Besides, the decreased lactic acid production in psoriatic plaques was restored to normal during the steroid treatment. The conversion of glucose into protein-bound amino acids seemed, however, to be uninfluenced by the steroid.

The results of the epithelial cell count in the different skin specimens are given in Table 12. Obviously, there is no difference between normal skin and uninvolved psoriatic skin. Hence, the increased respiration and protein formation in uninvolved psoriatic skin could not be due merely to an increased number of epithelial cells compared to normal skin. Moreover, the percentage of epithelial tissue was found to be somewhat lower in uninvolved psoriatic skin (61 %) than in normal skin (65 %).

Regarding the psoriatic plaques, the number of epithelial cells was significantly lower than in the other skin slices. Consequently, the higher percentage of epithelial tissue, found in the psoriatic plaques (77 %) seemed to be of little importance when evaluating the metabolic activity *per se* of the epidermis in psoriasis.

Table 10. *Utilization of specifically labeled glucose (Glucose-1-C^{14} and glucose-6-C^{14}) in normal and psoriatic skin slices.* Results expressed as μg of glucose converted per 100 mg of tissue (wet wt.) after 2 hours incubation at 37° C in pure O_2. Glucose concentration 0.2 %. Total radioactivity 20 μC per ml phosphate medium, pH 7.3.

Case	Exp. No.	Medium	CO_2		Lipids		Free amino acids			Protein-bound amino acids (total)	Lactic acid $\left(\frac{C\text{-}1}{C\text{-}6}\text{ ratio}\right)$
			μg	C-1/C-6	ST	FA	Asp. ac.	Glut. ac.	Alanine		
Normal skin	24	C-1	19	1.6	0.3	0.5	2	54	11		1.1
		C-6	12		0.3	0.4	2	55	23		
Normal skin	27	C-1	51	3.4	0.5	0.3	6	77	54		1.1
		C-6	15		0.5	0.3	7	72	45		
Normal skin	40	C-1	38	2.1	0.1	0.1	3	37	24	5	1.1
		C-6	18		0.4	0.2	4	22	15	10	
Normal skin	41	C-1	17	1.7	0.2	0.3		30	13	4	0.8
		C-6	10		0.3	0.3		37	19	5	
Psoriasis-plaque	26	C-1	91	2.4	5	3	3	20	15		1.0
		C-6	39		4	3	3	18	17		
Psoriasis-plaque	28	C-1	92	2.2	2	4	2	43	15		1.1
		C-6	42		2	4	4	47	23		
Psoriasis-plaque	44	C-1	79	2.0				24	2		0.9
		C-6	39					19	3		

Table 11. *The effects of systemical steroid treatment on the utilization of labeled glucose in psoriatic skin slices.*

A. Specifically labeled glucose. Glucose concentration 0.2 %. Total radioactivity 20 μC per ml medium.

Case	Exp. No.	Treatment	Incubation medium	O_2 μl	CO_2		Protein-bound amino acids (total in μg)	Lactic acid $\left(\frac{C\text{-}1}{C\text{-}6}\text{ ratio}\right)$
					μg	C-1/C-6		
Psoriasis-plaque	44	—	Glucose-1-C^{14}	197	79	2.0	—	1.1
			Glucose-6-C^{14}	188	39		32	
	45	Kenacort 4 mg × 4	Glucose-1-C^{14}	153	59	2.2	29	1.1
			Glucose-6-C^{14}	139	27		38	

Table 11.

B. Universally labeled glucose. Glucose concentration 0.1 %. Total radioactivity 20 μC per ml medium.

Case	Exp. No.	Treatment	Incubation medium	O_2 μl	CO_2 μg	Protein-bound amino acids (total in μg)	Lactic acid %[1]
Psoriasis-uninvolved skin	46	—	Glucose-U-C^{14}	112	58	—	91
	47	Kenacort 4 mg × 4		105	32	—	80
Psoriasis-uninvolved skin	51	—	Glucose-U-C^{14}	138	45	12	81
	52	Kenacort 4 mg × 4		99	39	14	79
Psoriasis-plaque	48	—	Glucose-U-C^{14}	239	90	26	63
	50	Kenacort 4 mg × 4		159	67	29	85
Psoriasis-plaque	53	—	Glucose-U-C^{14}	209	86	28	73
	54	Kenacort 4 mg × 4		159	85	25	81

[1] In per cent of the total uptake of glucose.

Table 12. *Epithelial cell count in normal and psoriatic skin slices.*

Results expressed per square mm of a cross section of the epithelial part of the different skin slices. The values are given as $\bar{x} \pm$ s.e.m. Number of experiments in parenthesis.

Group	Number of cells
I. Normal skin (10)	40.5 ± 2.7
II. Psoriasis-uninvolved skin (7)	42.6 ± 2.1
III. Psoriasis-plaque (7)	21.9 ± 2.2

t-Analysis of differences in cell count between the groups

I—II $t = 0.57$, $P < 0.7$
I—III $t = 4.99^{***}$, $P < 0.001$
II—III $t = 6.73^{***}$, $P < 0.001$

CHAPTER V

DISCUSSION

GENERAL CONSIDERATIONS ON THE METHOD EMPLOYED

The method employed in this work for elucidating the fate of labeled glucose in normal and in psoriatic skin slices involves many compromises. Firstly, the optimal conditions as regards temperature and composition of the incubation medium, required for successful Warburg experiments, cannot be regarded as physiologic. Second, *in vitro* experiments do not ensure a sufficient removal of metabolized products, which leads to an inevitable accumulation of certain intermediates. Third, the technique employed does not give any information on the specific activity of the different intermediates, separated mainly by chromatographic procedures. Hence, the data reported, *expressed as μg of glucose converted*, do not elucidate the total formation of carbon dioxide, lactic acid, amino acids and lipids.

Finally, the results of metabolic studies, conducted *in vitro*, may not simply be extrapolated to the intact living skin. Moreover, in this connection, it must be borne in mind that incubation experiments performed on skin slices from one part of the body cannot even be directly compared with similar experiments performed on skin specimens from another part of the same body. This statement is based on the finding, previously reported (53), that skin slices from different parts of the body have various metabolic rates. However, in the present study the skin samples were invariably taken from the gluteal region.

In spite of the limitations, summarized above, the technique employed has previously been successfully used for metabolic investigations on rat liver and brain slices (3, 5, 16), rat diaphragm muscle (6, 31), rat pituitary gland (4), rat brown adipose tissue (7), different parts of the rabbit brain (17) and different parts of the hypothalamus, pituitary gland and brain in goats (1, 30). The method has, moreover, also been used for an ingenious study of the effect of neuropharmaca on the glucose metabolism in different parts of the goat brain (46).

The basic principles of the entire technique, concerning the reproducibility of the counting and registration operations, has previously been thoroughly studied at the Istituto Superiore di Sanità in Rome (24).

OXYGEN UPTAKE AND CARBON DIOXIDE PRODUCTION

The considerably increased oxygen uptake and the abundantly enhanced CO^2 production in the diseased psoriatic skin is a dominant feature in the present investigation. These findings are in line with the increased respiration and the high dehydrogenase

activity in the psoriatic plaque, previously reported (12, 45, 74, 79, 82). Moreover, this appears to be an explanation of the well documented beneficial effect of cignolin, chrysarobin and various tar preparations on the psoriatic process (29, 36, 40, 42, 66, 71); all of which products may be regarded as reducing agents. Furthermore, it appears plausible to assume that when the keratinization process occurs in an oxidative environment, it leads to the formation of pathologic keratin containing an increased amount of oxidized disulfide bridges (See p. 11).

The increased oxygen consumption and carbon dioxide formation in *uninvolved psoriatic skin*, compared with normal skin, is another interesting aspect of the psoriatic process. The histologic studies of uninvolved skin of psoriatics, undertaken by *Madden* (49) several years ago, revealed a typical microscopic picture of psoriasis. Later on, it was possible to observe other peculiarities of the intact psoriatic skin: An abnormal reactive hyperemia response (52), a heightened electrical resistance (25) and an elongation and dilation of the capillaries (18). Besides, a peculiar, hitherto unidentified lipid soluble substance has been found in uninvolved skin from psoriatics (60). In this connection it should, furthermore, be pointed out that hydrolysis of the total proteins yielded the same qualitative distribution pattern of free amino acids in both uninvolved and diseased psoriatic skin (Fig. 14 and 15). Hence, the biochemical changes of the uninvolved skin of patients with psoriasis, indicated by the present study, support the concept that the entire skin is affected by the psoriatic process.

The incubation of normal and of psoriatic skin slices with specifically labeled glucose yielded a significantly higher C_1-CO_2 than C_6-CO_2 production in all samples. The $\frac{C_1}{C_6}$ ratio for carbon dioxide, thus found, was about 2, equal in normal and psoriatic skin slices. These values are comparable to those, recently reported for cartilage slices from rachitic rats (8), for pituitary tissue (19) and for hypothalamic tissue (30). As previously pointed out, a ratio of 1 would have been registered if the glucose was exclusively metabolized along the glycolytic and citric acid pathway (assuming that the triose phosphates are freely interconvertible). The findings of the present investigation, thus indicate that nontriose pathways, probably the HMP-shunt, operate in the interconversion of glucose in normal and psoriatic skin.

The remaining data given in Table 10 may, however, in this connection be regarded as contradictory. Concurrent glucose catabolism along the HMP-shunt would namely result in a greater transfer of C^{14} to alanine from C-6 of glucose than from C-1, since C-1 here is lost as CO_2 (9). The equal amounts of lipids, free amino acids and lactic acid found by simultaneous incubation of human skin slices in specifically labeled glucose may therefore indicate that most of the glucose is metabolized along the EMP. With regard to the abundantly increased carbon dioxide formation in the psoriatic plaque, the HMP-shunt must here, nevertheless, be considered to play an important role. Hence, the amounts of glucose obviously catabolized along this pathway facilitate the biosynthesis of pentoses, and consequently, the production of nucleotides, which are utilized by the rapidly proliferating epidermal cells of the psoriatic skin. Moreover, the activity of the HMP-shunt in psoriasis provides an increased amount of TPNH, which

possibly may be used for biosynthesis of lipids (83), thus explaining the increased lipogenesis in the psoriatic epidermis.

LACTIC ACID FORMATION

From the results of the present study it can be demonstrated that the major part of the assimilated glucose was converted into lactic acid. The abundant production of lactic acid in human skin slices resembles the values, previously reported, for skin slices (44), the hypothalamus and pituitary gland in goats (1), rat liver and diaphragm muscle (31) and different parts of rabbit brain (17).

Finally, it ought to be emphasized that no spontaneous glycolysis occurred in the substrate during incubation; this is illustrated in Fig. 9. This autoradiogram was obtained after chromatographic separation of the substrate, incubated both with and without tissue. In the latter case the incubation medium was used instead of water in the thermobarometer ("TB").

In this connection it must be pointed out that the radioactivity of the incubation medium, stored in a refrigator at —20° C, was continuously checked and found to be constant.

AMINO ACID AND PROTEIN FORMATION

The two-dimensional chromatographic analysis of the tissue extract, after incubation of human skin slices, revealed a large number of different C^{14}-amino acids. The distribution pattern of the free amino acids thus obtained, included not only all of the amino acids previously recorded by incubation of brain and liver slices (15, 17) but also serine and glycine. It should be emphasized in this connection that GABA was almost invariably found in the tissue extract and the incubation medium. Moreover, the radiochromatograms of the tissue extract revealed most of the C^{14}-intermediates belonging to the Krebs cycle; whereas previous investigators in this field have recorded only trace amounts of these substances (15).

The quantitative evaluation of the two-dimensional radiochromatograms of the aqueous ethanolic tissue extract was complicated by the fact that the different free amino acids in varying degree also were found in the incubation medium. This phenomenon, which apparently has not been recorded in previous investigations on different animal biopsy material, where the same technique was applied, may probably be due to the genuine excretory nature of the skin. At any rate, in all the samples, glutamic acid, alanine and aspartic acid were evidently the dominant feature in the quantitative distribution pattern of the free amino acids. These findings justify the statement that the transamination reactions play an important role in the biology of the human skin, thus facilitating the biosynthesis of essential amino acids.

In rat diaphragm *Chain et al.* found that the insoluble residue was composed mainly of fibrous material and a water-insoluble C^{14}-glycogen fraction (15). This finding, however, could not be confirmed by the present investigation, where the insoluble

residue showed high radioactivity due to the biosynthesis of C^{14}-proteins and C^{14}-lipids. These results are thus in better agreement with the conditions recorded in rat pituitary gland where the radioactivity of the insoluble residue was found to be present mainly in the protein (4).

Hydrolysis of the C^{14}-proteins, after incubation of normal and of psoriatic skin slices with C^{14}-glucose, invariably yielded a uniform distribution pattern of four amino acids: Aspartic acid, glutamic acid, alanine and leucine-isoleucine. Hence, no C^{14}-amino acid, specific for the psoriatic skin process, was observed. This finding is in line with the similar amino acid composition in normal and in psoriatic skin, previously reported (39). The formation of protein-bound amino acids was, however, abundantly increased in the psoriatic plaque. This observation, which indicates an increased rate of epidermal proliferation in psoriasis, has recently been confirmed by the recording of an accelerated glycine-C^{14} incorporation into the proteins of stratum corneum in psoriatics (63).

LIPID FORMATION

The considerably increased lipid formation in the psoriatic plaques, which is illustrated in Table 9, represents one of the most characteristic features of the present work. The total amount of C^{14}-lipids, derived from C^{14}-glucose, in the psoriatic skin was, however, notably small in comparison with the quantities of glucose converted into lactic acid, CO_2 or amino acids.

The enhanced lipid formation in psoriasis may, as previously pointed out, be due to the HMP-shunt activity in the psoriatic skin, which, according to *Tepperman and Tepperman* (83), facilitates the formation of TPNH, and consequently, also the biosynthesis of fatty acids and sterols. The high lipogenic potential of the psoriatic skin may finally be coupled with the increased lipid content of the psoriatic horny layer, detected by histochemical and chemical methods (12, 88) and with the finding of crystalline cholesterol in the psoriatic scales, reported by *Swanbeck* (80).

MISCELLANEOUS COMMENTS

Very little is known about the mode of action of steroid hormones, in spite of the increasing use of these drugs, not least of all in dermatology. Since, in recent years, the value of oral steroid therapy in psoriasis has been well documented (51, 68) it seemed important to conduct a preliminary study on this subject using the radiopaperchromatographic technique. The choice of steroid hormones was influenced by the fact that Kenacort has been considered to be the most effective drug in the treatment of psoriasis (51).

The unchanged $\frac{C_1}{C_6}$ ratio for carbon dioxide after Kenacort treatment, indicating that steroid hormones, in contrast to ACTH (83), do not increase the oxidation of glucose along the HMP-shunt, is in line with previous investigations performed on liver slices

(2). The marked decrease of the oxygen uptake and carbon dioxide formation during steroid treatment partly confirm the suggestion, recently reported (89), that the mode of action of steroid hormones mainly involves an impaired respiration and a decreased protein formation. Concerning the production of protein-bound amino acids during Kenacort treatment, which can be seen in Table 11, it should be pointed out, however, that the values only represent the amount of labeled glucose converted. Thus, nothing can be stated about the total amount of proteins present.

CHAPTER VI

SUMMARY

1. In the present investigation the quantitative radiopaperchromatographic technique, developed by *Chain et al.*, was, for the first time, applied to human skin slices.
2. The results of this investigation primarily indicated that this technique constitutes a valuable tool for studying the metabolism in human skin, thus opening up new ways not readily accessible by other methods.
3. The oxygen uptake and the carbon dioxide production were considerably increased in diseased psoriatic skin compared with normal skin or uninvolved skin from psoriatics. The importance of these findings, with regard to the management of psoriasis and the defective keratinization process in the psoriatic skin is briefly discussed.
4. The increased oxygen uptake and carbon dioxid formation in uninvolved psoriatic skin, compared with normal skin, supports the hypothesis, previously based mainly on histologic examinations, that the skin *as a whole* takes part in the psoriatic process. It therefore seems adequate to introduce the term "latent psoriasis" (20), defined as a state, which exists before the development of clinical psoriasis and wherein probably some metabolic defect is present.
5. The incubation of normal and psoriatic skin slices with specifically labeled glucose (Glucose-1-C^{14} and glucose-6-C^{14}) revealed that the HMP-shunt operates in the interconversion of glucose in normal as well as in psoriatic skin. The importance of the metabolism of glucose along the HMP-shunt in psoriasis is briefly discussed.
6. The lactic acid produced, generally accounted for 75—90 per cent of the total uptake of glucose. The abundant lactic acid formation in human skin is in line with the previously reported results, obtained by incubation of different tissues using the same technique. The production of lactic acid, expressed in per cent of the total uptake of glucose, was, however, significantly lower in psoriatic plaques than in normal skin or uninvolved psoriatic skin.
7. The free amino acid formation in normal and in psoriatic skin slices was found to involve the conversion of glucose into aspartic acid, glutamic acid, glutamine, serine, glycine, alanine, GABA and proline.
8. The quantitative distribution pattern of the free amino acids was dominated by glutamic acid, alanine and aspartic acid, indicating the important role of the transamination reactions in the biology of the skin.
9. Incubation of psoriatic skin slices during different time intervals showed that the formation of protein-bound amino acids is an approximately linear function due to a successive building up of the C^{14}-amino acids into proteins.

10. The radiochromatograms of the aqueous ethanolic tissue extract, after incubation of normal and psoriatic skin slices, revealed many of the intermediates belonging to the Krebs cycle (Citric acid, maleic acid, alfa-ketoglutaric acid and succinic acid).
11. The insoluble residue obtained after incubation of normal and of psoriatic skin slices with C^{14}-glucose was found to contain a significant amount of radioactivity due to the biosynthesis of C^{14}-proteins and C^{14}-lipids.
12. After hydrolysis of the insoluble residue, the radiochromatograms revealed in all the samples a uniform distribution pattern of four amino acids: Aspartic acid, glutamic acid, alanine and leucine-isoleucine. The formation of protein-bound amino acids was abundantly increased in the psoriatic plaque compared with that in normal skin or uninvolved psoriatic skin.
13. The biosynthesis of C^{14}-lipids, separated into sterols and fatty acids, was considerably increased in diseased psoriatic skin compared with normal skin or uninvolved psoriatic skin.
14. The effects of systemical steroid treatment on the metabolism of specifically and universally labeled glucose in psoriatic skin slices was found to involve mainly a decreased oxygen consumption and an impaired carbon dioxide formation.

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APPENDIX

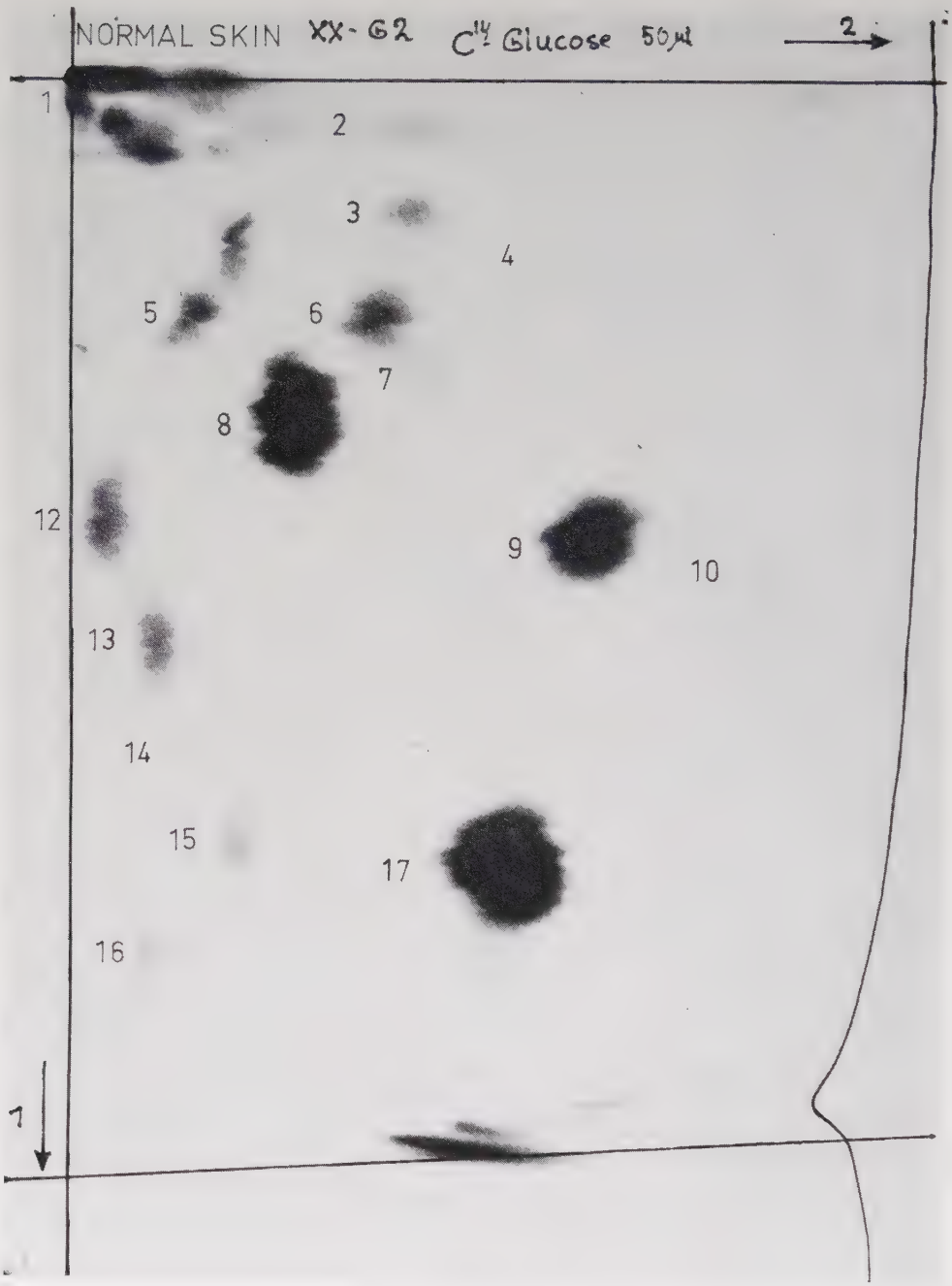

Fig. 6. *Autoradiochromatogram of the aqueous ethanolic tissue extract after incubation of normal skin with* C^{14}*-glucose.*

Two-dimensional chromatogram developed in solvent No. 1 (↓) and solvent No. 2 (→). For identification of the different spots see Table 5.

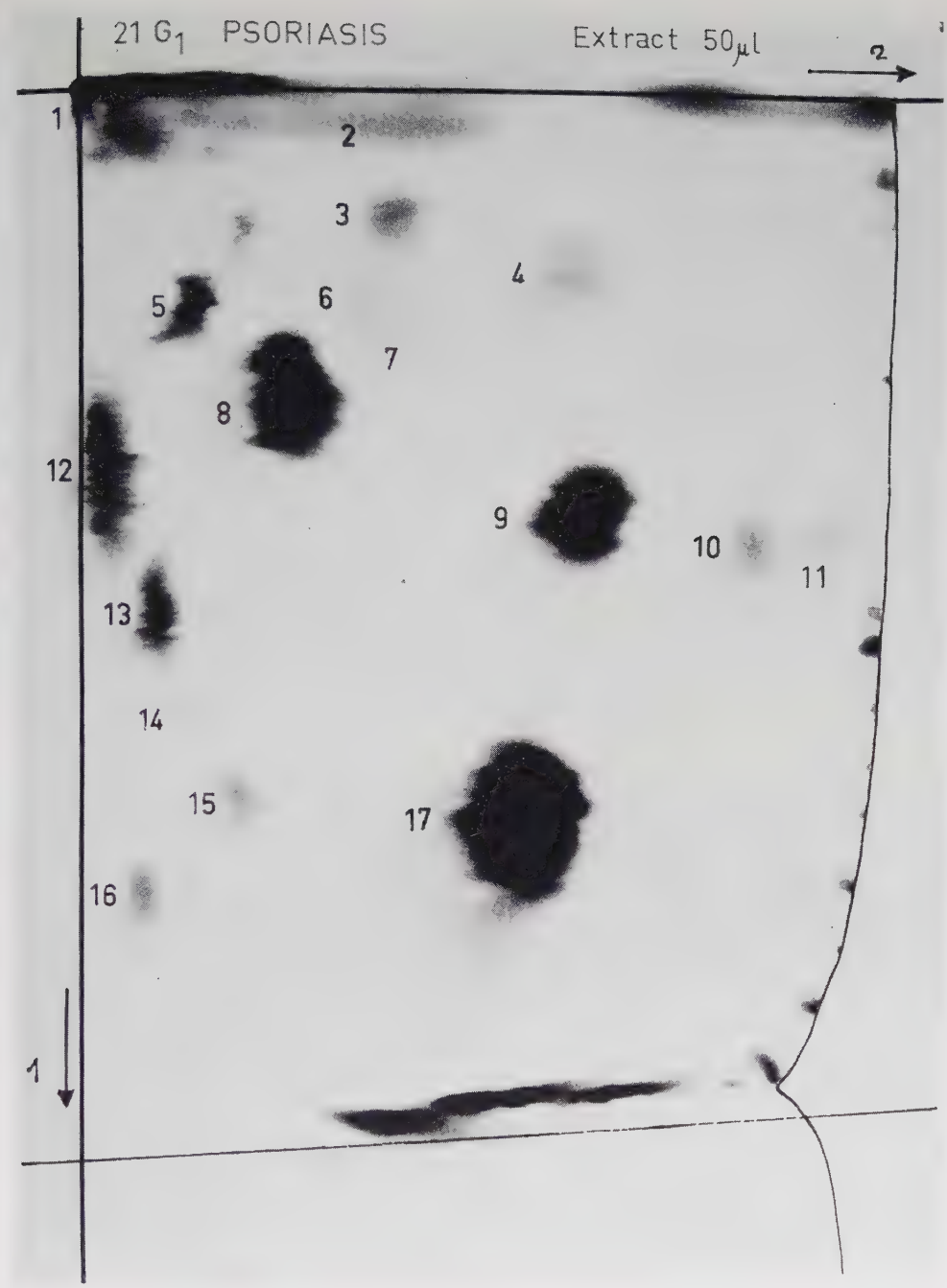

Fig. 7. *Autoradiochromatogram of the aqueous ethanolic tissue extract after incubation of diseased psoriatic skin with C^{14}-glucose.*
Two-dimensional chromatogram developed in solvent No. 1 (↓) and solvent No. 2 (→). For identification of the different spots see Table 5.

Fig. 8. *Autoradiochromatogram of the incubation medium after incubation of normal skin with C^{14}-glucose. One-dimensional chromatogram developed in solvent No. 3.*

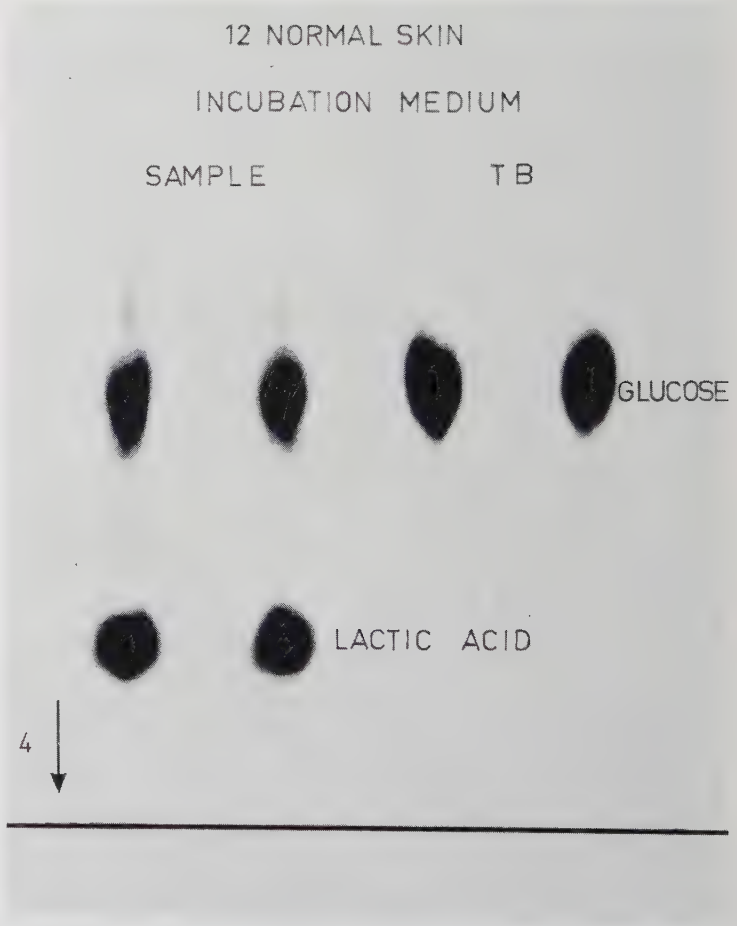

Fig. 9. *Autoradiochromatogram of the incubation medium after incubation of normal skin with C^{14}-glucose. One-dimensional chromatogram developed in solvent No. 4.*

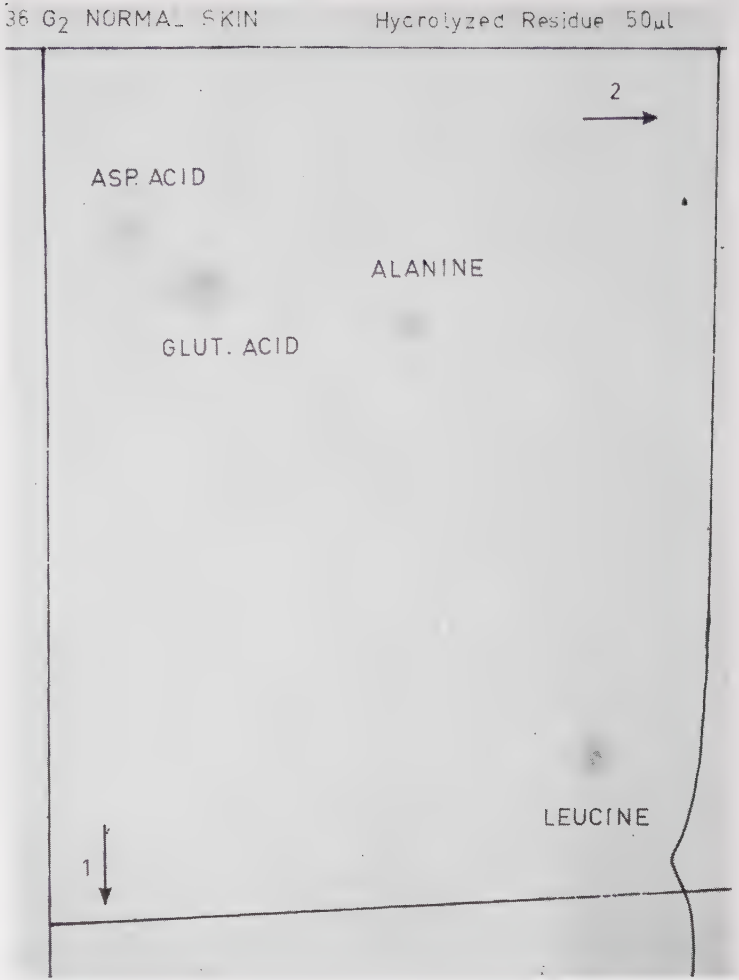

Fig. 10. *Autoradiochromatogram of the aqueous ethanolic extract of the hydrolyzed residue after incubation of normal skin with C^{14}-glucose. Two-dimensional chromatogram developed in solvent No. 1 (↓) and solvent No. 2 (→). For identification of the different spots see Table 5.*

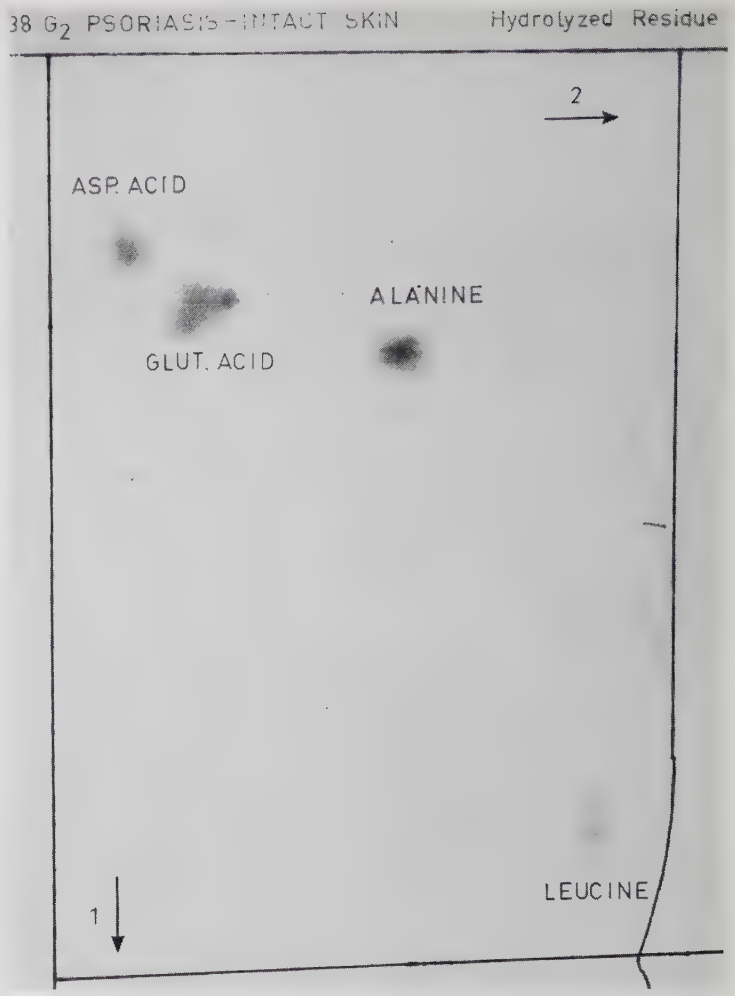

Fig. 11. *Autoradiochromatogram of the aqueous ethanolic extract of the hydrolyzed residue after incubation of uninvolved psoriatic skin with C^{14}-glucose. Two-dimensional chromatogram developed in solvent No. 1 (↓) and solvent No. 2 (→). For identification of the different spots see Table 5.*

Fig. 12. *Autoradiochromatogram of the aqueous ethanolic extract of the hydrolyzed residue after incubation of diseased psoriatic skin with C^{14}-glucose. Two-dimensional chromatogram developed in solvent No. 1 (↓) and solvent No. 2 (→). For identification of the different spots see Table 5.*

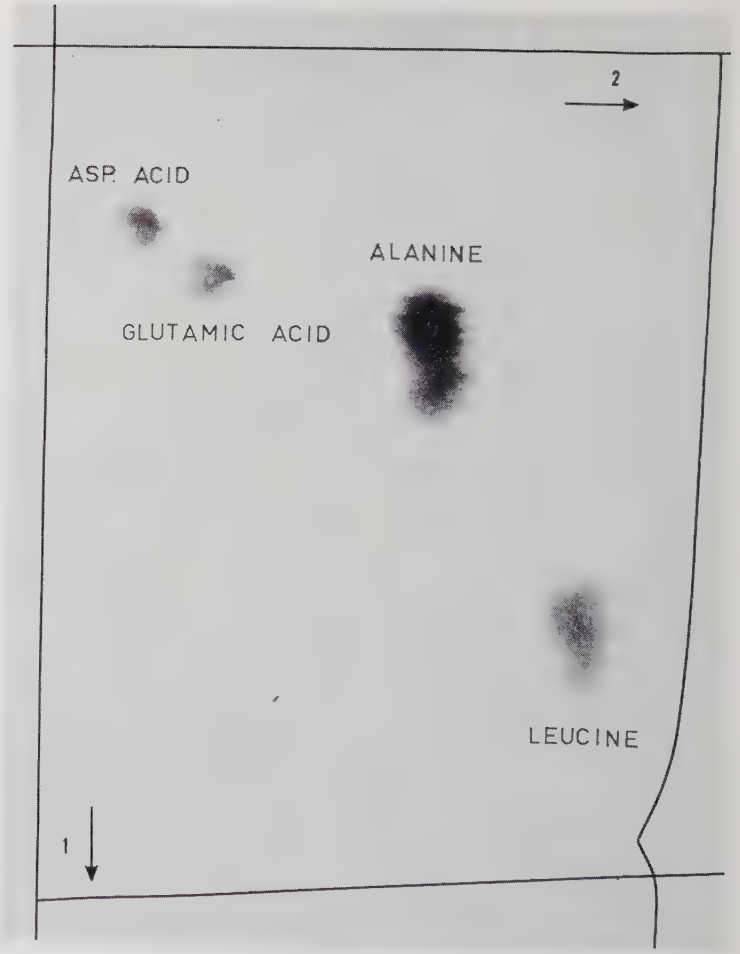

Fig. 13. *Two-dimensional chromatogram, developed in solvent No. 1 and 2, of a reference solution. This solution contained aspartic acid, glutamic acid, alanine and leucine. The spots are developed and coloured by spraying with a ninhydrin solution.*

Fig. 14. *Two-dimensional chromatogram of the aqueous ethanolic extract of the hydrolyzed residue after incubation of diseased psoriatic skin with glucose. The hydrolyzed amino acids are developed by spraying with a ninhydrin solution.*

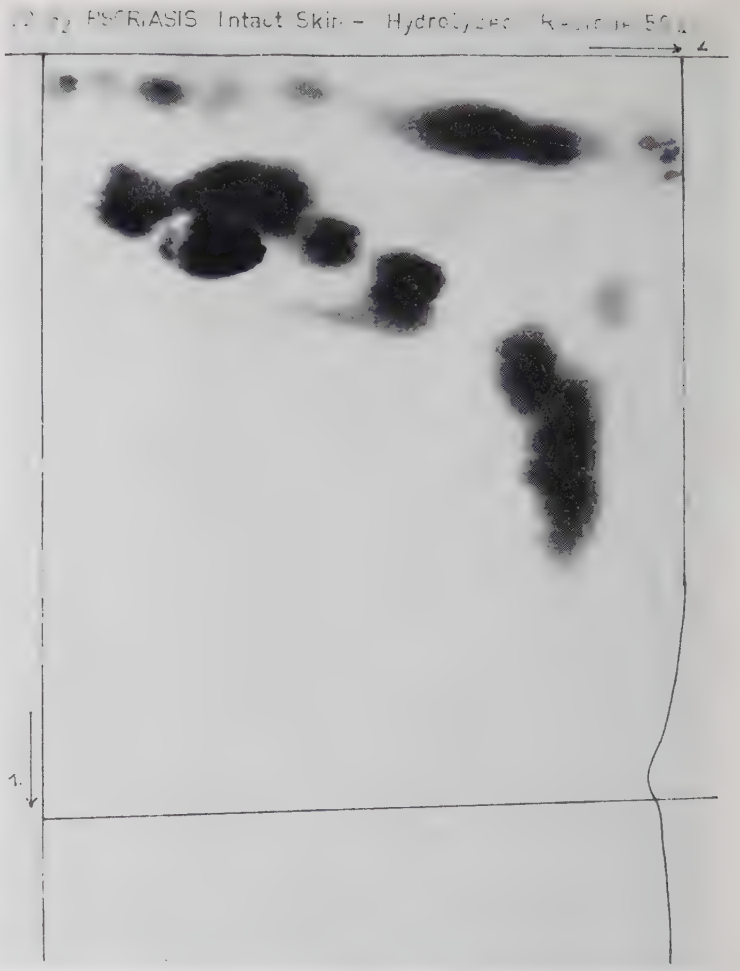

Fig. 15. *Two-dimensional chromatogram of the aqueous ethanolic extract of the hydrolyzed residue after incubation of uninvolved psoriatic skin with glucose. The hydrolyzed amino acids are developed by spraying with a ninhydrin solution.*

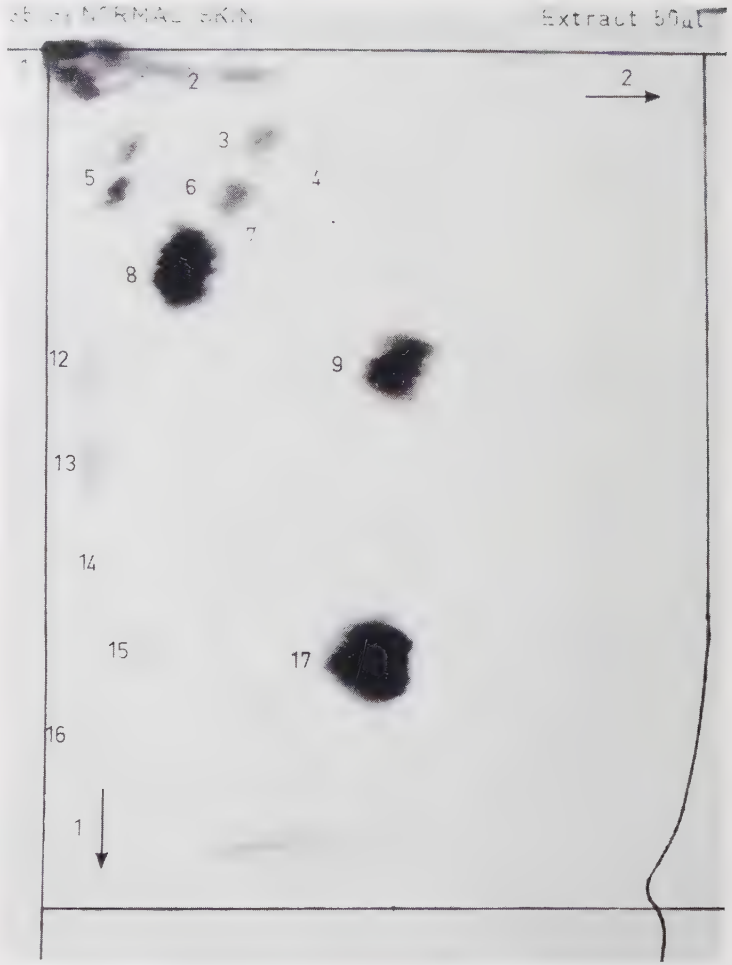

Fig. 16. *Autoradiochromatogram of the aqueous ethanolic tissue extract after incubation of normal skin with C^{14}-glucose.*
Two-dimensional chromatogram developed in solvent No. 1 (↓) and solvent No. 2 (→). For identification of the different spots see Table 5.

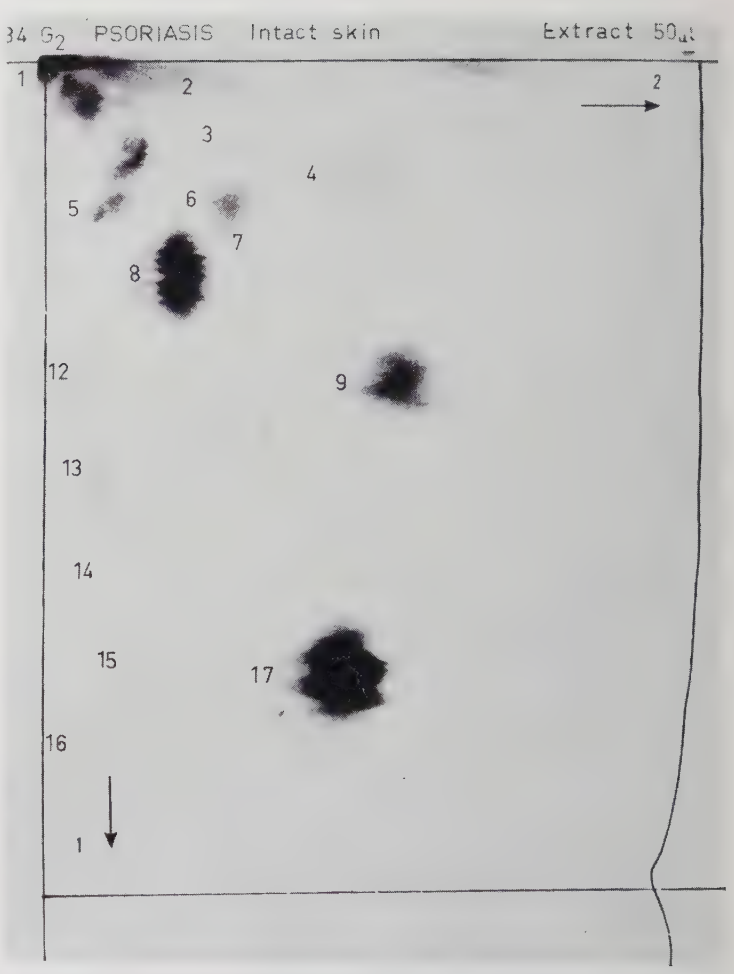

Fig. 17. *Autoradiochromatogram of the aqueous ethanolic tissue extract after incubation of uninvolved psoriatic skin with C^{14}-glucose.*
Two-dimensional chromatogram developed in solvent No. 1 (↓) and solvent No. 2 (→). For identification of the different spots see Table 5.

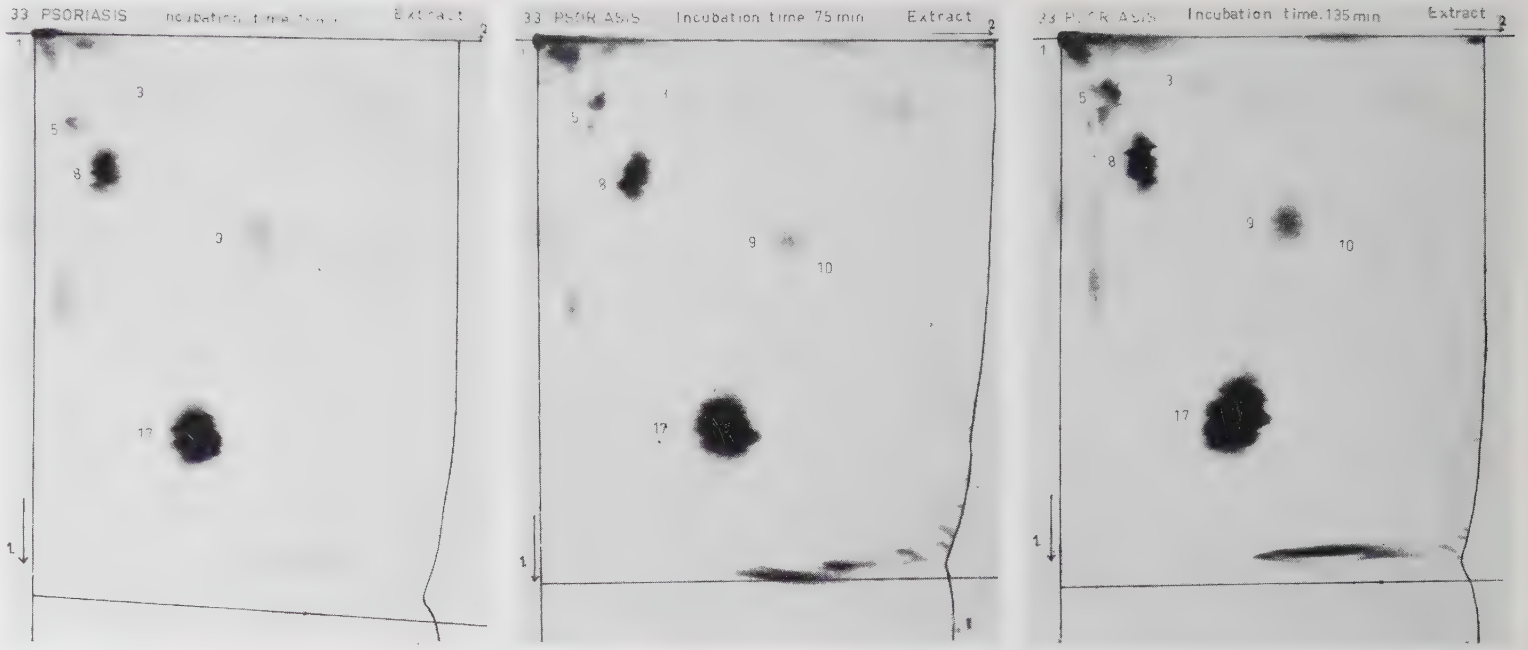

Fig. 18. *Autoradiochromatograms of the aqueous ethanolic tissue extract after incubation of diseased psoriatic skin with C^{14}-glucose for 15 minutes (A), 75 minutes (B) and 135 minutes (C).*

Two-dimensional chromatograms developed in solvent No. 1 (↓) and solvent No. 2 (→). For identification of the different spots see Table 5.

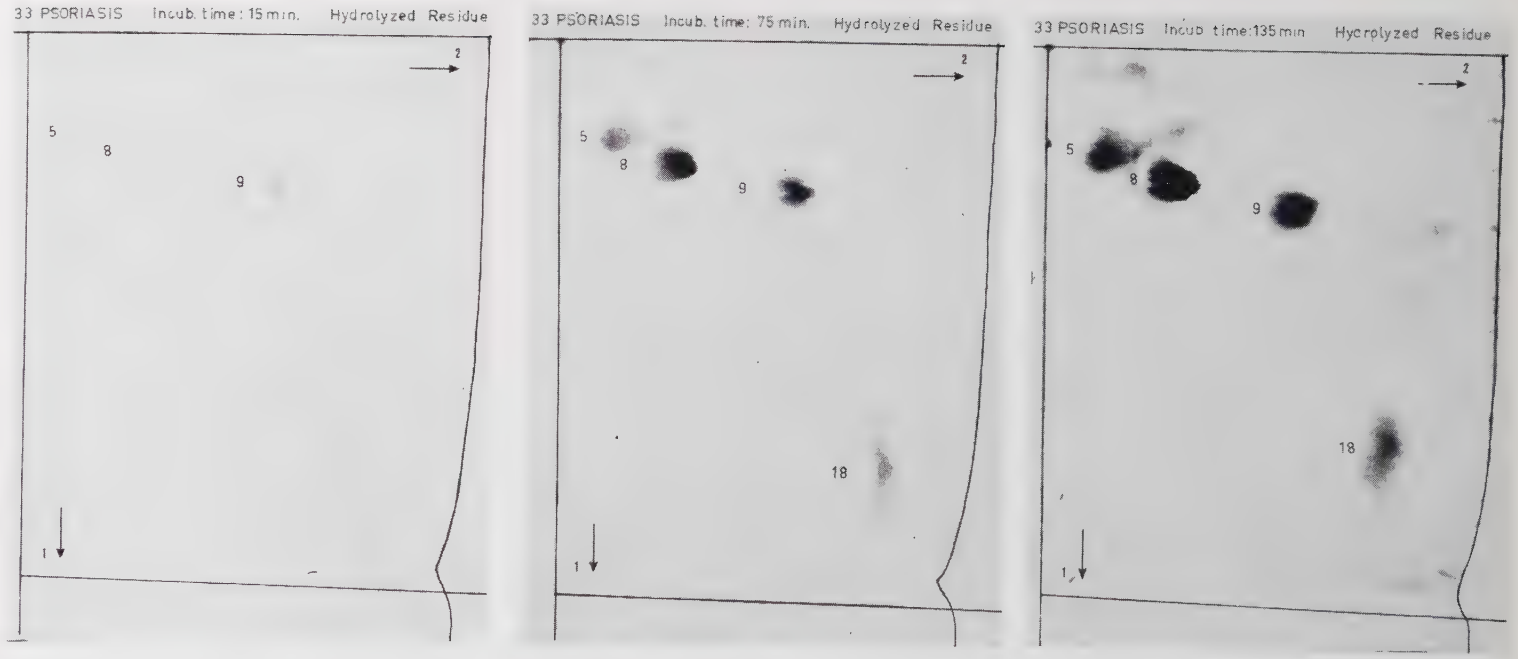

Fig. 19. *Autoradiochromatograms of the aqueous ethanolic extract of the hydrolyzed residue after incubation of diseased psoriatic skin with C^{14}-glucose for 15 minutes (A), 75 minutes (B) and 135 minutes (C).*

Two-dimensional chromatograms developed in solvent No. 1 (↓) and solvent No. 2 (→). For identification of the different spots see Table 5.

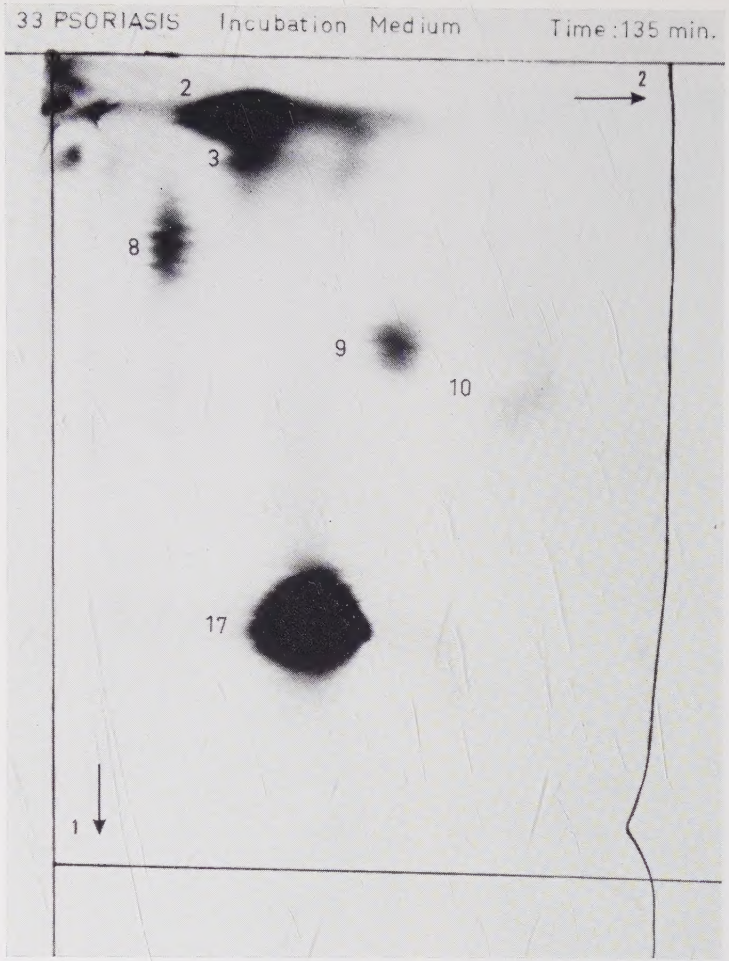

Fig. 20. *Autoradiochromatograms of the incubation medium after incubation of psoriatic skin with C^{14}-glucose.*
Two-dimensional chromatogram developed in solvent No. 1 (↓) and solvent No. 2 (→). For identification of the different spots see Table 5.

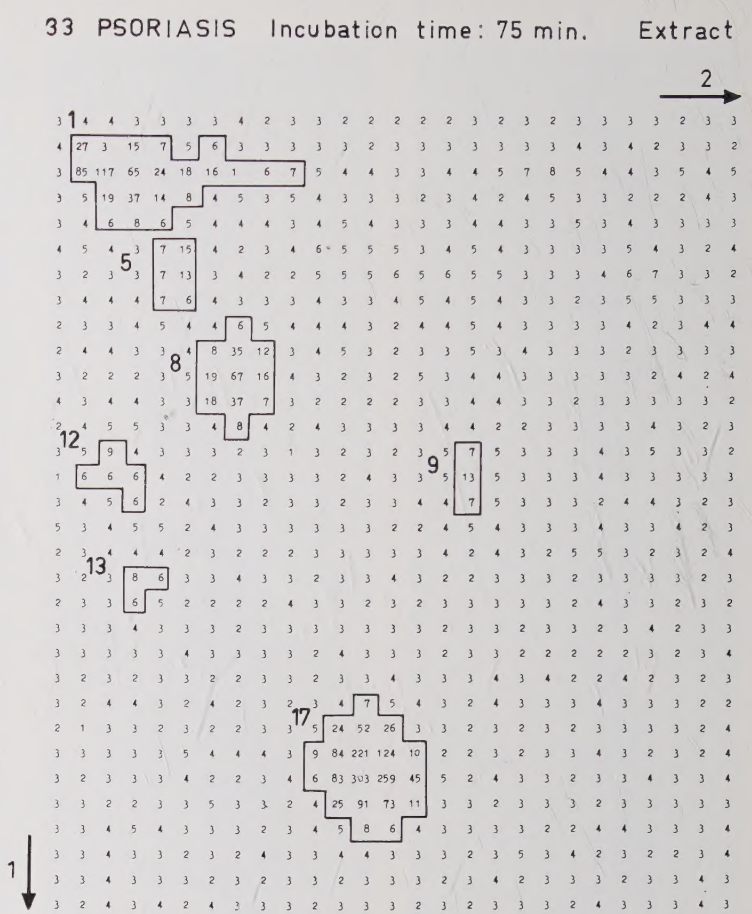

Fig. 21. *Direct automatic scanning of a radiochromatogram of the tissue extract after incubation of diseased psoriatic skin with C^{14}-glucose for 75 minutes (=Fig. 18B). Two-dimensional chromatogram developed in solvent No. 1 (↓) and solvent No. 2 (→). For identification of the different spots see Table 5.*

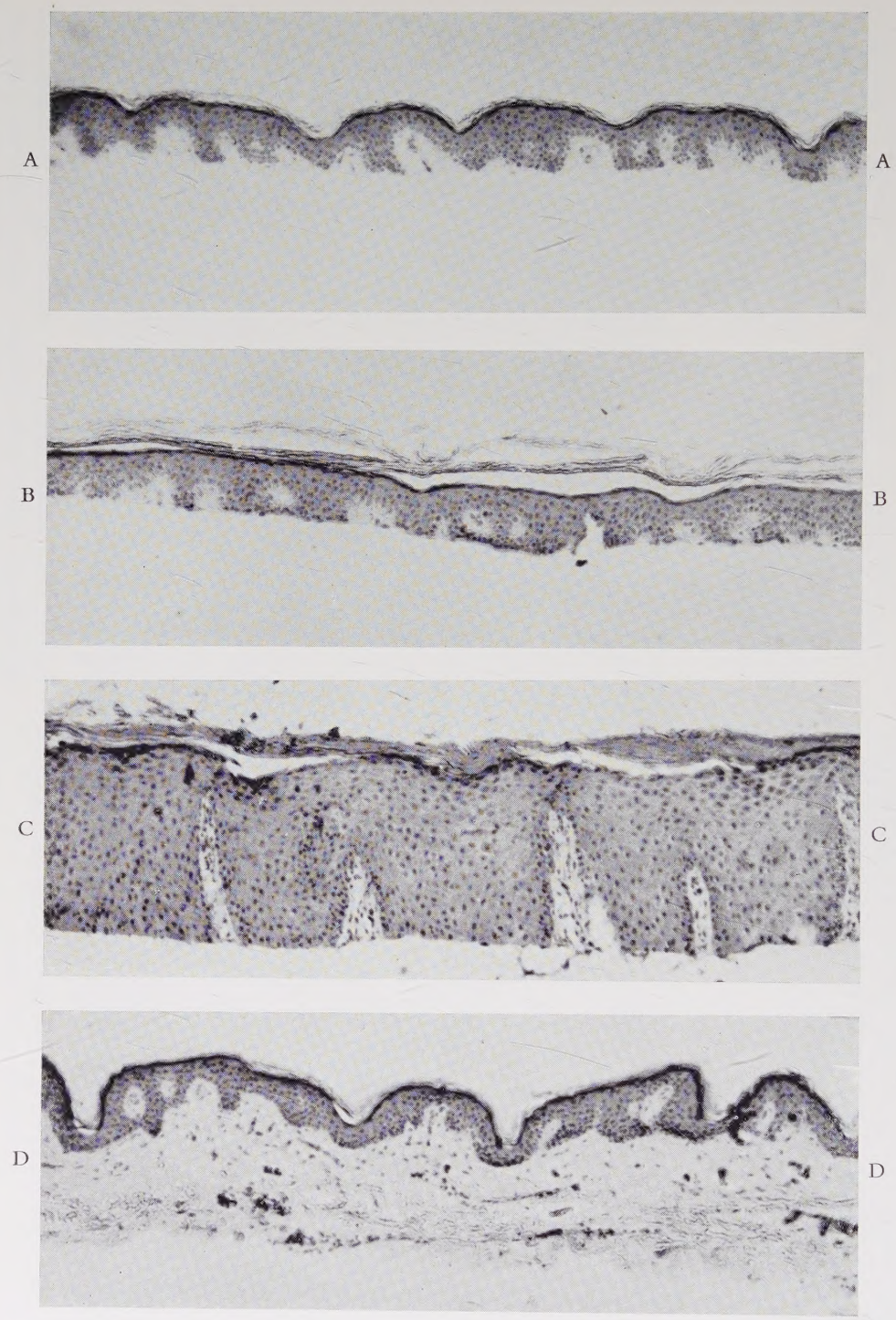

Fig. 22. *Microphotographs of skin sections removed by means of the Brown Electro-Dermatome. A Normal skin. B Uninvolved psoriatic skin. C Diseased psoriatic skin (Plaque). C Uninvolved psoriatic skin (Epidermal-dermal skin section);* ×125.